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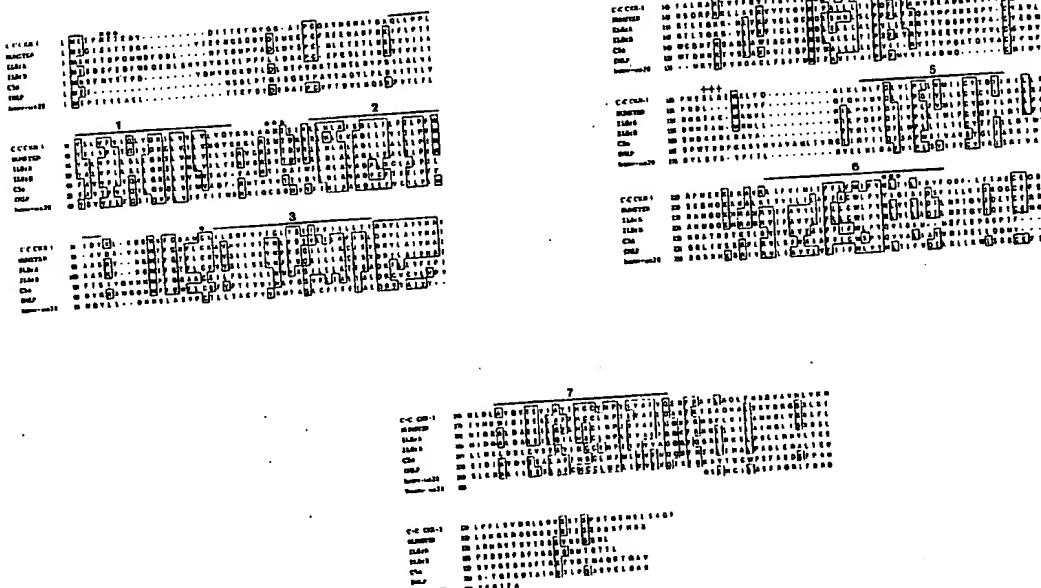
(57) Abstract

DNA isolates encoding the human C-C chemokine receptor C-C CKR-1 and methods of obtaining such DNA are provided, together with expression systems for recombinant production of C-C CKR-1 useful in therapeutic or diagnostic compositions. Additionally, a method for identifying new chemokine receptors is provided.

C-C CKR-1, C-C CHEMOKINE RECEPTOR

This invention relates to the field of cytokine
receptors, their antibodies, and their use as diagnostic
5 and therapeutic agents.

BACKGROUND OF THE INVENTION

Cytokines are biological molecules which affect
10 inflammatory and immune-related effector cells. The
inflammatory cytokines, or "chemokines," have a variety of
biological properties including selective leukocyte
chemotaxis and activation. These chemokines form a
superfamily, denoted in the literature alternatively as the
15 PF4 superfamily or the intercrines, that has been divided
into two classes based on whether the first two conserved
cysteine residues are separated by an intervening amino
acid (C-X-C, or α), or whether they are adjacent (C-C, or
β). The C-X-C class members include, for example,
20 interleukin-8 (IL-8), melanocyte growth stimulating factor
(MGSA), and platelet factor 4 (PF4), while the C-C class
includes RANTES (Regulated on Activation, Normal T
Expressed and Secreted) and monocyte chemotactic peptide-1
(MCP-1). The C-X-C class exerts proinflammatory activity
25 mainly through their action on neutrophils, whereas the C-C
class appears to be monocyte chemoattractants.
Much attention has been focused on receptor/ligand
interactions in this superfamily, with more data being
available on C-X-C than C-C chemokine binding. It has
30 become clear that chemokine receptor/ligand interactions on
target inflammatory cells seem to be strictly regulated.
For example, no cross competition for binding sites has
been observed on either monocytes or neutrophils between
members of the C-X-C or C-C branches (Leonard, E. J. et al.
35 Immunol. Today 11:97-101, 1990; Samanta, A. K. et al. J.
Exp. Med. 169:1185-1189, 1989; Yoshimura, T. et al. J.
Immunol. 145:292-7, 1990), consistent with the differential
chemoattractant effects on these two cell types. Direct
binding data for the C-C chemokines is surprisingly sparse.

2

Human MCP-1 has been reported to bind to monocytes with an affinity of about 2 nM, with no sites detectable on neutrophils (Valente, A. J. et al. Biochem. Biophys. Res. Commun. 176:309-14, 1991; Yoshimura, T. et al. J. Immunol.
5   145:292-7, 1990). A single report shows human Act-2, a human MIP-1β (HuMIP-1β) variant, binding to between 7,000 and 45,000 sites on PBMC with an affinity of between 7.8 and 12 nM (Napolitano, M. et al. J. Exp. Med. 172:285-289, 1990). Kwon and colleagues have characterized the binding
10   of murine MIP-1α on a mouse T cell and a macrophage cell line, finding a K_d of 1.5 and 0.9 nM, respectively (Oh, K. O. et al. J. Immunol. 147:2978-83, 1991).

Most of the molecular details regarding leukocyte motility remain to be elucidated. Recently, however, the
15   receptors for the anaphylatoxin C5a (Gerard, N. P. et al. Nature 349:614-7, 1991), the bacterial formylated tripeptide fMLP (Boulay, F. et al. Biochemistry 29:11123-11133, 1990), and the C-X-C chemokine IL-8 (Holmes, W. E. et al. Science 253:1278-80, 1991; Murphy, P. M. et al.
20   Science 253:1280-3, 1991) have been cloned using molecular techniques. All of these receptors display amino acid sequences which are predicted to conform to an architecture containing seven-transmembrane-spanning segments connected by a series of intra- and extracellular loops. The primary
25   sequences of these receptors revealed domains which were conserved in receptors associated with cell motility, but not in other seven-transmembrane-spanning receptors.

Accordingly, it is an object of the invention to provide isolated C-C chemokine receptor (C-C CKR-1) for
30   use as a therapeutic or diagnostic reagent.

It is another object of the invention to make variants of C-C CKR-1 for use as antagonists or agonists.

It is another object of the invention to generate antibodies against C-C CKR-1 for use as diagnostic and
35   therapeutic agents.

It is another object of the invention to provide a method for identifying new chemokine receptors.

3

SUMMARY OF THE INVENTION

One aspect of the invention is the isolation of the
novel chemokine receptor, C-C CKR-1.

In another aspect, the invention provides a
5   composition comprising C-C CKR-1 that is free of
contaminating polypeptides of the animal species from which
the C-C CKR-1 is derived.

In another aspect of the invention, C-C CKR-1, or
fragments thereof (which also may be synthesized by
10   chemical methods), is fused (by recombinant expression or
in vitro covalent methods) to an immunogenic polypeptide
and this fusion polypeptide, in turn, is used to immunize
an animal to raise antibodies against a C-C CKR-1 epitope.
Anti-C-C CKR-1 antibodies are recovered from the serum of
15   immunized animals. Alternatively, monoclonal antibodies
are prepared from cells of the immunized animal in
conventional fashion.

Another aspect of the invention is the use of anti-C-C
CKR-1 antibodies in the diagnosis of (in vitro or in vivo)
20   or (when immobilized on an insoluble matrix) the
purification of chemokine receptors which bind thereto.

Another aspect of the invention is the derivatization
of C-C CKR-1 in vitro to prepare immobilized C-C CKR-1 and
labeled C-C CKR-1, particularly for purposes of diagnosis
25   of C-C CKR-1 or its antibodies, or for affinity
purification of C-C CKR-1 antibodies themselves.

Another aspect of the invention is the formulation of
C-C CKR-1, its derivatives, or its antibodies into
physiologically acceptable vehicles, especially for
30   therapeutic use. Such vehicles include sustained-release
formulations of C-C CKR-1.

In still other aspects, the invention provides an
isolated nucleic acid molecule encoding C-C CKR-1, labeled
or unlabeled, and a nucleic acid sequence that is
35   complementary to, or hybridizes under defined conditions to
a nucleic acid sequence encoding C-C CKR-1.

In addition, the invention provides a replicable
vector comprising the nucleic acid molecule encoding C-C
CKR-1 operably linked to control sequences recognized by a

4

host transformed by the vector; host cells transformed with
the vector; and a method of using a nucleic acid molecule
encoding C-C CKR-1 to effect the production of C-C CKR-1,
comprising expressing the nucleic acid molecule in a
5   culture of the transformed host cells and recovering C-C
CKR-1 from the host cell culture. The nucleic acid
sequence is also useful in hybridization assays for C-C
CKR-1 nucleic acid.

       Another aspect of the invention is substitutional,
10   deletional, or insertional variants of C-C CKR-1 amino
acids and/or glycosyl residues, including variants having
non-native glycosylation. These variants are prepared by
in vitro or recombinant methods. Sequence variants are
optionally screened for immuno-cross-reactivity with C-C
15   CKR-1 and for C-C CKR-1 antagonist or agonist activity.

       Another aspect of the invention is a method for
identifying new C-C chemokine receptors.

       Another aspect of the invention is a method for
determining the biological activity of a C-C chemokine
20   variant on C-C CKR-1, by transforming a host cell with DNA
encoding C-C CKR-1, culturing the host cell to express the
receptor on its surface, harvesting the cells, contacting
the cells with a C-C chemokine variant, and determining the
biological activity of the variant on the receptor.

25   In further embodiments, the invention provides
transgenic animals comprising C-C CKR-1 from another
species, animals in which C-C CKR-1 is expressed in a
tissue in which it is not ordinarily found, or animals in
which C-C CKR-1 is inactivated, by, for example, gene
30   disruption.

BRIEF DESCRIPTION OF THE FIGURES

       Figure 1 (SEQ ID NOS:1-7) depicts the predicted amino
acid sequence of C-C CKR-1, and alignment of the predicted
35   amino acid sequence with HUMSTSR (Genbank accession
#M99293), IL8rA (Holmes, W. E. et al. Science 253:1278-80,
1991), IL8rB (Murphy, P. M. et al. Science 253:1280-3,
1991), C5a receptor (Gerard, N. P. et al. Nature 349:614-7,
1991), fMLP receptor (Boulay, F. et al. Biochemistry

5

29:11123-11133, 1990) and the open reading frame of
cytomegalovirus, US28. The seven putative transmembrane
spanning domains are overlined. Glycosylation sites are
indicated with black dots above the sequence. The two
5   cysteine residues implicated in disulfide bonding are
indicated with asterisks. The consensus site for protein
kinase C phosphorylation is indicated as "+". Conserved
amino acids appearing more then two times in the alignment
are boxed.

10   Figure 2 depicts Northern blot analysis of RNA from
hematopoietic cells probed with C-C CKR-1. 5 µg of poly A$^+$
or 20 µg of total RNA from the cell lines indicated was
size fractionated on 1% formaldehyde-agarose, transferred
to nitrocellulose and hybridized with radiolabeled C-C CKR-
15   1 cDNA. The filter was washed with 0.5X SSC, 0.1% SDS at
55°C and the autoradiograph was developed after 4-6 hours
exposure at -70°C with intensifying screens. RNA molecular
weight markers were also run on the gel and are indicated
on the side.

20   Figures 3A and 3B depict Southern blot analysis of
human genomic DNA probed with C-C CKR-1. In Figure 3A, 10
µg of genomic DNA was digested with the restriction enzyme
indicated, run on a 0.6% agarose gel, blotted onto
Genescreen® and hybridized with radiolabeled C-C CKR-1
25   cDNA. The filter was washed with 0.5X SSC, 1% SDS at 55°C
and the autoradiograph was developed after overnight
exposure at -70°C with intensifying screens. DNA molecular
weight markers were also run on the gel and are indicated
on the side. In Figure 3B, the same blot was washed more
30   stringently with 0.2X SSC, 0.1% SDS at 60°C and
autoradiography performed as indicated before.

       Figures 4A and 4B are graphs depicting intracellular
Ca^{++} concentrations of 293 cells transfected with C-C CKR-1
cDNA and challenged with human MIP-1α(HuMIP-1α) and RANTES.
35   In Figure 4A, 50% confluent cells were transfected with 10-
20 µg of plasmid DNA by the calcium-phosphate precipitation
method. After transient expression for 12-24 hours, cells
were harvested, loaded with the calcium probe INDO-1 AM and

6

assayed by spectrofluorometric methods at 37°C with
continuous stirring. Various concentrations of HuMIP-1α,
as indicated, were added after 12 seconds. The
intracellular concentrations of Ca^{++} was determined as

5   described (Naccache, P. H. et al. J. Immunol. 142:2438-44,
1989). In Figure 4B, details were as for Figure 4A, except
that various concentration of RANTES, as indicated, were
used.

Figures 5A-5D are graphs depicting desensitization in

10   response to the challenge of the same or different ligands
by 293 cells transiently expressing C-C CKR-1. Details are
as described in Figure 4. The transfected cells were first
challenged at 12 seconds with 100 nM of HuMIP-1α or 250 nM
of RANTES, and then at 70 seconds with the same

15   concentration of ligands in the order indicated.

Figures 6A and 6B are graphs depicting the binding of
^{125}I-HuMIP-1α and ^{125}I-RANTES on 293 cells transfected with
C-C CKR-1 cDNA. In Figure 6A, Human embryonic kidney cells
(293 cells) were transfected with 10-20 µg plasmid DNA as

20   described in Figure 4. Transfected cells were incubated
for 2 hours at 4°C with ^{125}I-HuMIP-1α in the presence of
increasing concentrations of unlabeled HuMIP-1α. The inset
shows Scatchard analysis of the binding data and revealed a
K_d of 5.1±0.3nM for ^{125}I-MIP-1α to C-C CKR-1. Figure 6B

25   depicts displacement of ^{125}I-RANTES with unlabeled HuMIP-1α
on 293 cells transfected with the C-C CKR-1 cDNA.
Scatchard analysis of the binding data revealed a K_d of
7.6±1.5nM for the displacement of ^{125}I-RANTES to the C-C
CKR-1.

30   Figure 7 is a graph depicting displacement of ^{125}I-
HuMIP-1α binding to 293 cells transfected with C-C CKR-1
cDNA. Cells were transfected as outlined in Figure 4 and
incubated for 2 hours at 4°C with ^{125}I-HuMIP-1α in the
presence of increasing concentrations of the cross

35   competing ligands, HuMIP-1α, murine MIP-1α, HuMIP-1β, MCP-1
and IL-8. The K_d and the number of sites, shown in the
bottom left corner, were determined by Scatchard analysis
of the binding data.

Figure 8 is a graph depicting the intracellular Ca^{++} concentration of 293 cells transiently expressing C-C CKR-1 and challenged with HuMIP-1α, RANTES, HuMIP-1β and MCP-1. Details are as described in Figure 4.

5   Figure 9 (SEQ ID NO:8) is the nucleotide sequence of C-C CKR-1 and its 3' noncoding region.

Figure 10 is a graph depicting the binding of radiolabeled HuMIP-1α to 293 cells transfected with the coding region of the open reading frame US28 in the

10   cytomegalovirus (CMV) genome. 293 cells were transfected with an expression construct containing the coding sequence of US28 in the sense or antisense orientation. After 12 hours, the cells were harvested and incubated with 0.9 nM of ^{125}I-HuMIP-1α in combination with 1 μM of either

15   unlabeled HuMIP-1α, murine MIP-1β, MCP-1, RANTES, or IL-8. The amount of displaceable ^{125}I-HuMIP-1α was determined by subtracting the amount of ^{125}I-HuMIP-1α bound in the absence of any cold ligand from the amount bound in the presence of cold ligand. Background refers to counts

20   obtained from cells transfected with the antisense orientation of US28.

DETAILED DESCRIPTION OF THE INVENTION
A. Definitions

25

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims.

"C-C CKR-1" is the chemokine receptor described infra

30   together with its amino acid sequence or cellular analogs, alleles, predetermined amino acid sequence mutations, glycosylation variants, and covalent modifications. Embodiments of C-C CKR-1 exclude known chemokine receptors, in particular those which are set forth in the Background

35   section above, and chemokine receptors statutorily obvious from those such chemokine receptors.

"Orphan receptor" is defined as the predicted polypeptide encoded by nucleic acid which hybridizes under low stringency conditions to probes designed from known

cytokine receptor nucleic acid sequences or other known
sequences likely to have structural similarity to cytokine
receptors, or detectable by PCR primers so designed,
wherein the predicted polypeptide is not previously known
5 in the art.

"C-C CKR-1 qualitative biological activity" is defined
as immunological cross-reactivity with at least one epitope
of purified C-C CKR-1.

"Immunologically cross-reactive" is intended to mean
10 that the candidate polypeptide is capable of competitively
inhibiting the binding of native C-C CKR-1 to polyclonal
antibodies or antisera raised against native C-C CKR-1,
respectively.

"Isolated C-C CKR-1 nucleic acid or polypeptide" is a
15 C-C CKR-1 nucleic acid or polypeptide that is identified
and separated from at least one contaminant (nucleic acid
or polypeptide respectively) with which it is ordinarily
associated in nature, such as from the human source of C-C
CKR-1 nucleic acid or polypeptide. In preferred
20 embodiments, C-C CKR-1 will be isolated to pharmaceutically
acceptable levels of purity with respect to proteins of its
species of origin. In preferred embodiments, C-C CKR-1
protein will be purified (1) to greater than 95% by weight
of protein, and most preferably more than 99% by weight,
25 (2) to a degree sufficient to obtain at least 15 residues
of N-terminal or internal amino acid sequence by an amino
acid sequenator commercially available on the filing date
hereof, or (3) to homogeneity by conventional nonreducing
SDS polyacrylamide gel electrophoresis (SDS-PAGE) using
30 Coomassie blue or, preferably, silver stain. Isolated C-C
CKR-1 includes C-C CKR-1 in situ within recombinant cells
which do not ordinarily express the C-C CKR-1 in question,
since, in this instance, at least one component of C-C CKR-
1 natural environment will not be present. Isolated C-C
35 CKR-1 includes C-C CKR-1 in a recombinant cell culture of
another species than the species of origin of the C-C CKR-1
since the C-C CKR-1 in such circumstances will be devoid of
source polypeptides. Ordinarily, however, isolated C-C
CKR-1 will be prepared by at least one purification step.

9

Isolated C-C CKR-1 nucleic acid includes a nucleic
acid that is identified and separated from at least one
containment nucleic acid with which it is ordinarily
associated in the natural source of the C-C CKR-1 nucleic
5   acid. Isolated C-C CKR-1 nucleic acid thus is present in
other than in the form or setting in which it is found in
nature. However, isolated C-C CKR-1-encoding nucleic acid
includes C-C CKR-1 nucleic acid in ordinarily C-C CKR-1-
expressing cells where the nucleic acid is in a chromosomal
10   location different from that of natural cells or is
otherwise flanked by a different DNA sequence than that
found in nature.

The nucleic acid or polypeptide may be labeled for
diagnostic and probe purposes, using a label as described
15   and defined further below in the discussion of diagnostic
assays.

"C-C CKR-1 nucleic acid" is defined as RNA or DNA
(a) containing at least 25 bases of the genomic or cDNA
sequence that encodes C-C CKR-1, (b) is complementary to
20   the genomic or cDNA sequence that encodes C-C CKR-1, (c)
which hybridizes to such nucleic acid and remains stably
bound to it under stringent conditions, or (d) encodes a
polypeptide sharing at least 50% sequence identity over the
entire length of the polypeptide, preferably at least 60%,
25   and more preferably at least 70%, with the amino acid
sequence of C-C CKR-1, and which polypeptide has the
ability to bind at least one C-C chemokine. Preferably the
hybridizing RNA or DNA contains at least 25 bases, more
preferably 40, and more preferably 60 bases which are
30   identical to the sequences encoding the C-C CKR-1 described
infra. Optimally, C-C CKR-1 nucleic acid consists
essentially only of sequence encoding C-C CKR-1 or the
complement of such sequences.

"Stringency" conditions for hybridization are defined
35   by washing conditions after the hybridization reaction.
Typically, hybridization conditions are defined as
employing overnight incubation at 42°C, in a solution
comprising 20% formamide, 5X SSC (150 mM NaCl, 15 mM
trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5X

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Denhardt's solution, 10% dextran sulfate, and 20 μg/ml
denatured, sheared salmon sperm DNA. "High stringency"
conditions for washing are defined as typically employing
0.2X SSC, 0.1% SDS at 55°C, while "low stringency"
5   conditions for washing are defined as typically employing
0.5X SSC, 1% SDS at 42°C. These conditions are well known
in the art. See, for example, Current Protocols in
Molecular Biology, eds. Ausubel, et al., Greene Publishing
Associates, NY, 1989.
10   The term "control sequences" refers to DNA sequences
necessary for the expression of an operably linked coding
sequence in a particular host organism. The control
sequences that are suitable for prokaryotes, for example,
include a promoter, optionally an operator sequence, and a
15   ribosome binding site. Eukaryotic cells are known to
utilize promoters, polyadenylation signals, and enhancers.
     Nucleic acid is "operably linked" when it is placed
into a functional relationship with another nucleic acid
sequence. For example, DNA for a presequence or secretory
20   leader is operably linked to DNA for a polypeptide if it is
expressed as a preprotein that participates in the
secretion of the polypeptide; a promoter or enhancer is
operably linked to a coding sequence if it affects the
transcription of the sequence; or a ribosome binding site
25   is operably linked to a coding sequence if it is positioned
so as to facilitate translation. Generally, "operably
linked" means that the DNA sequences being linked are
contiguous and, in the case of a secretory leader,
contiguous and in reading phase. However enhancers do not
30   have to be contiguous. Linking is accomplished by ligation
at convenient restriction sites. If such sites do not
exist, then synthetic oligonucleotide adapters or linkers
are used in accord with conventional practice.
     The starting plasmids used to practice this invention
35   are commercially available, are publicly available on an
unrestricted basis, or can be constructed from such
available plasmids in accord with published procedures. In
addition, other equivalent plasmids are known in the art
and will be apparent to the ordinary artisan. Methods for

restriction enzyme digestion, recovery or isolation of DNA, hybridization analysis, and ligation are conventional and by this time well known to the ordinary artisan. Similarly, the cell lines used to practice this invention

5   are commercially available or are publicly available on an unrestricted basis.

Another method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Engels et al. (Agnew, _Chem. Int. Ed. Engl._

10   28:716-734, 1989). These methods include triester, phosphite, phosphoramidite and H-phosphonate methods, typically proceeding by oligonucleotide synthesis on solid supports.

"Recovery" or "isolation" of a given fragment of DNA

15   from a restriction digest means separation of the digest on polyacrylamide or agarose gel by electrophoresis, identification of the fragment of interest by comparison of its mobility versus that of marker DNA fragments of known molecular weight, removal of the gel section containing the

20   desired fragment, and separation of the gel from DNA. This procedure is known generally. For example, see Sambrook, et al., _Molecular Cloning: A Laboratory Manual_, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989).

Amino acids are referred to by their standard three

25   letter IUPAC abbreviations.

B. General Methods for Practicing the Invention

1. Preparation of Native C-C CKR-1 Nucleic Acid

The use of the singular article "the" with respect to

30   C-C CKR-1 is not intended to suggest that only one DNA sequence encodes C-C CKR-1. In fact, it is expected that alleles, processing intermediates, and predetermined sequence variants described infra will vary in sequence from the DNA encoding native C-C CKR-1. Further, C-C CKR-1

35   may fall within a subfamily of chemokine receptors having a high degree of sequence homology but which vary sufficiently as to not constitute alleles. All of these sequences fall within the ambit of C-C CKR-1 nucleic acid.

DNA sequences encoding C-C CKR-1 may be either genomic

12

or cDNA. Any representative genomic library may be
screened with the probes described below. Methods for
genomic DNA preparation and the construction of cDNA
libraries are well known in the art. See, for example,
5   Sambrook et al., supra.

        2. Amino Acid Sequence Variants of C-C CKR-1
        Amino acid sequence variants of C-C CKR-1 are prepared
by introducing appropriate nucleotide changes into C-C CKR-
10 1 DNA, or by in vitro synthesis of the desired C-C CKR-1
polypeptide. Such variants include, for example, deletions
from, or insertions or substitutions of, residues within
the amino acid sequence of native C-C CKR-1. Any
combination of deletion, insertion, and substitution can be
15 made to arrive at the final construct, provided that the
final construct possesses the desired characteristics.
        The amino acid changes also may alter post-
translational processing of C-C CKR-1, such as changing the
number or position of glycosylation sites or by altering
20 its membrane anchoring characteristics.
        In designing amino acid sequence variants of C-C CKR-
1, the location of the mutation site and the nature of the
mutation will depend on C-C CKR-1 characteristic(s) to be
modified. The sites for mutation can be modified
25 individually or in series, e.g., by (1) substituting first
with conservative amino acid choices and then with more
radical selections depending upon the results achieved, (2)
deleting the target residue, or (3) inserting residues of
the same or a different class adjacent to the located site,
30 or combinations of options 1-3.
        A useful method for identification of certain residues
or regions of C-C CKR-1 polypeptide that are preferred
locations for mutagenesis is called "alanine scanning
mutagenesis" as described by Cunningham and Wells (Science
35 244:1081-1085, 1989). Here, a residue or group of target
residues are identified (e.g., charged residues such as
arg, asp, his, lys, and glu) and replaced by a neutral or
negatively charged amino acid (most preferably alanine or
polyalanine) to affect the interaction of the amino acids

13

with the surrounding aqueous environment in or outside the
cell. Those domains demonstrating functional sensitivity
to the substitutions then are refined by introducing
further or other variants at or for the sites of
5   substitution. Thus, while the site for introducing an amino
acid sequence variation is predetermined, the nature of the
mutation per se need not be predetermined. For example, to
optimize the performance of a mutation at a given site,
alanine scanning or random mutagenesis may be conducted at
10 the target codon or region and the expressed C-C CKR-1
variants are screened for the optimal combination of
desired activity.

C-C CKR-1 variants will exhibit at least a biological
activity of the parental sequence, for example, chemokine
15 binding or antigenic activity. Preferably, the
antigenically active C-C CKR-1 is a polypeptide that binds
to an antibody raised against the polypeptide in its native
conformation, "native conformation" generally meaning the
polypeptide as found in nature which has not been denatured
20 by chaotropic agents, heat or other treatment that
substantially modifies the three dimensional structure of
the polypeptide (this can be determined, for example, by
migration on nonreducing, nondenaturing sizing gels).
Antibody used in determination of antigenic activity is
25 rabbit polyclonal antibody raised by formulating the native
non-rabbit polypeptide in Freund's complete adjuvant,
subcutaneously injecting the formulation, and boosting the
immune response by intraperitoneal injection of the
formulation until the titer of anti-polypeptide antibody
30 plateaus.

Amino acid sequence deletions generally range from
about 1 to 30 residues, more preferably about 1 to 10
residues, and typically are contiguous. Preferably,
deletions are made in regions of the protein that are the
35 least conserved when C-C CKR-1 amino acid sequence is
compared with other chemokine receptors. Such deletions
will be more likely to modify the biological activity of
the polypeptides more significantly than deletions made
elsewhere. The number of consecutive deletions will be

14

selected so as to preserve the tertiary structure of C-C
CKR-1 in the affected domain, e.g., beta pleated sheet or
alpha helix.

Amino acid sequence insertions include amino- and/or
5   carboxyl-terminal fusions ranging in length from one
residue to polypeptides containing a hundred or more
residues, as well as intrasequence insertions of single or
multiple amino acid residues. Intrasequence insertions
(i.e., insertions within C-C CKR-1 sequence) may range
10   generally from about 1 to 10 residues, more preferably 1 to
5, most preferably 1 to 3.

Insertional variants of C-C CKR-1 or its extracellular
segments include the fusion to the N- or C-terminus of C-C
CKR-1 of immunogenic polypeptides, e.g., bacterial
15   polypeptides such as β-lactamase or an enzyme encoded by
the E. coli trp locus, or yeast protein, and C-terminal
fusions with proteins having a long half-life such as in
place of V_H or V_C domains of immunoglobulins comprising
constant regions, albumin, or ferritin (for example, as
20   described in WO 89/02922, published 6 April 1989).

Another group of variants are amino acid substitution
variants. These variants have at least one amino acid
residue in C-C CKR-1 molecule removed and a different
residue inserted in its place. The sites of greatest
25   interest for substitutional mutagenesis include sites
identified as the active site(s) of C-C CKR-1, and sites
where the amino acids found in C-C CKR-1 from various
species are substantially different in terms of side-chain
bulk, charge, and/or hydrophobicity.

30   Other sites of interest are those in which particular
residues of C-C CKR-1 are conserved when compared with
other chemokine receptors. These positions may be
important for the biological activity of C-C CKR-1. These
sites, especially those falling within a sequence of at
35   least three other identically conserved sites, are
substituted in a relatively conservative manner. Such
conservative substitutions are shown in Table I under the
heading of preferred substitutions. If such substitutions
result in a change in biological activity, then more

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substantial changes, denominated exemplary substitutions in
Table I, or as further described below in reference to
amino acid classes, are introduced and the products
screened.

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<u>Table I</u>

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	ser	ser
Arg (R)	lys; gln; asn; ala	lys
Asn (N)	gln; his; lys; arg; ala; asp	asp
Asp (D)	glu; asn; ala	asn
Cys (C)	ser; ala; val	ala
Gln (Q)	asn; glu; ala	asn; glu
Glu (E)	asp; gln; ala	gln
Gly (G)	ala; asn	ala
His (H)	asn; gln; lys; arg; ala	asn
Ile (I)	leu; val; met; ala; phe	val
Leu (L)	ile; val; met; ala; phe	met
Lys (K)	arg; gln; asn; met; ala	arg
Met (M)	leu; phe; ile;ala	leu
Phe (F)	leu; val; ile; ala; tyr	leu
Pro (P)	ala	ala
Ser (S)	thr; ala	ala
Thr (T)	ser; val; ala	ser
Trp (W)	tyr; phe; ala	tyr
Tyr (Y)	trp; phe; thr; ala; gln	phe
Val (V)	ile; leu; met; phe; ala; thr	ala

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35   Substantial modifications in function or immunological
identity of C-C CKR-1 are accomplished by selecting
substitutions that differ significantly in their effect on
maintaining (a) the structure of the polypeptide backbone
in the area of the substitution, for example, as a sheet or

40 helical conformation, (b) the charge or hydrophobicity of
the molecule at the target site, or (c) the bulk of the

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side chain. Naturally occurring residues are divided into
groups based on common side chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

5 (3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro;
and

(6) aromatic: trp, tyr, phe.

10   Non-conservative substitutions will entail exchanging
a member of one of these classes for another. Such
substituted residues may be introduced into regions of C-C
CKR-1 that are homologous with other chemokine receptors,
or, more preferably, into the non-homologous regions of the

15 molecule.

Any cysteine residues not involved in maintaining the
proper conformation of C-C CKR-1 may be substituted,
generally with serine, to improve the oxidative stability
of the molecule and prevent aberrant cross-linking.

20   DNA encoding amino acid sequence variants of C-C CKR-1
is prepared by a variety of methods known in the art.
These methods include, but are not limited to, isolation
from a natural source (in the case of naturally occurring
amino acid sequence variants) or preparation by

25 oligonucleotide-mediated (or site-directed) mutagenesis,
PCR mutagenesis, and cassette mutagenesis of an earlier
prepared variant or a non-variant version of C-C CKR-1.
These techniques may utilize C-C CKR-1 nucleic acid (DNA or
RNA), or nucleic acid complementary to C-C CKR-1 nucleic

30 acid. Oligonucleotide-mediated mutagenesis is a preferred
method for preparing substitution, deletion, and insertion
variants of C-C CKR-1 DNA. This technique is well known in
the art (see, for example, as described by Adelman et al.,
DNA 2:183, 1983). PCR mutagenesis is also suitable for

35 making amino acid variants of C-C CKR-1 (see Erlich, supra,
pp. 61-70). Another method for preparing variants, cassette
mutagenesis, is based on the technique described by Wells
et al. (Gene 34:315-323, 1985).

3.   Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding native or variant C-C
CKR-1 is inserted into a replicable vector for further
cloning (amplification of the DNA) or for expression. Many
5 vectors are available, and selection of the appropriate
vector will depend on (1) whether it is to be used for DNA
amplification or for DNA expression, (2) the size of the
DNA to be inserted into the vector, and (3) the host cell
to be transformed with the vector. Each vector contains
10 various components depending on its function (amplification
of DNA or expression of DNA) and the host cell for which it
is compatible. The vector components generally include,
but are not limited to, one or more of the following: a
signal sequence, an origin of replication, one or more
15 marker genes, an enhancer element, a promoter, and a
transcription termination sequence.

a.   Signal Sequence Component

In general, a signal sequence may be a component of
20 the vector, or it may be a part of C-C CKR-1 DNA that is
inserted into the vector.

b.   Origin of Replication Component

Both expression and cloning vectors contain a nucleic
25 acid sequence that enables the vector to replicate in one
or more selected host cells. Generally, in cloning vectors
this sequence is one that enables the vector to replicate
independently of the host chromosomal DNA, and includes
origins of replication or autonomously replicating
30 sequences. Such sequences are well known for a variety of
bacteria, yeast, and viruses. The origin of replication
from the plasmid pBR322 is suitable for most Gram-negative
bacteria, the 2μ plasmid origin is suitable for yeast, and
various viral origins (SV40, polyoma, adenovirus, VSV or
35 BPV) are useful for cloning vectors in mammalian cells.
Generally, the origin of replication component is not
needed for mammalian expression vectors (the SV40 origin
may typically be used only because it contains the early
promoter).

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Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in E. coli

5   and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host

10 genome. This is readily accomplished using Bacillus species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in Bacillus genomic DNA. Transfection of Bacillus with this vector results in homologous recombination with the genome

15 and insertion of C-C CKR-1 DNA. However, the recovery of genomic DNA encoding C-C CKR-1 is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise C-C CKR-1 DNA.

20   c.   Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium.

25 Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement

30 auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for Bacilli.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are

35 successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et al., J. Molec. Appl. Genet. 1:327-341, 1982), mycophenolic acid (Mulligan et al.,

19

Science 209:1422-1427, 1980) or hygromycin (Sugden et al.,
Mol. Cell. Biol. 5:410-413, 1985). The three examples
given above employ bacterial genes under eukaryotic control
to convey resistance to the appropriate drug G418 or
5   neomycin (geneticin), xgpt (mycophenolic acid), or
hygromycin, respectively.

Another example of suitable selectable markers for
mammalian cells are those that enable the identification of
cells competent to take up C-C CKR-1 nucleic acid, such as
10   dihydrofolate reductase (DHFR) or thymidine kinase. The
mammalian cell transformants are placed under selection
pressure which only the transformants are uniquely adapted
to survive by virtue of having taken up the marker.
Selection pressure is imposed by culturing the
15   transformants under conditions in which the concentration
of selection agent in the medium is successively changed,
thereby leading to amplification of both the selection gene
and the DNA that encodes C-C CKR-1. Amplification is the
process by which genes in greater demand for the production
20   of a protein critical for growth are reiterated in tandem
within the chromosomes of successive generations of
recombinant cells. Increased quantities of C-C CKR-1 are
synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection
25   gene are first identified by culturing all of the
transformants in a culture medium that contains
methotrexate (Mtx), a competitive antagonist of DHFR. An
appropriate host cell when wild-type DHFR is employed is
the Chinese hamster ovary (CHO) cell line deficient in DHFR
30   activity, prepared and propagated as described by Urlaub
and Chasin, _Proc. Natl. Acad. Sci. U.S.A._, 77(7):4216-4220,
1980). The transformed cells are then exposed to increased
levels of methotrexate. This leads to the synthesis of
multiple copies of the DHFR gene, and, concomitantly,
35   multiple copies of other DNA comprising the expression
vectors, such as the DNA encoding C-C CKR-1. This
amplification technique can be used with any otherwise
suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding
the presence of endogenous DHFR if, for example, a mutant

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DHFR gene that is highly resistant to Mtx is employed (EP
117,060). Alternatively, host cells (particularly wild-
type hosts that contain endogenous DHFR) transformed or co-
transformed with DNA sequences encoding C-C CKR-1, wild-

5   type DHFR protein, and another selectable marker such as
aminoglycoside 3' phosphotransferase (APH) can be selected
by cell growth in medium containing a selection agent for
the selectable marker such as an aminoglycosidic
antibiotic, e.g., kanamycin, neomycin, or G418.

10   A suitable selection gene for use in yeast is the trp1
gene present in the yeast plasmid YRp7 (Stinchcomb et al.,
Nature 282:39-43, 1979); Kingsman et al., Gene 7:141-152,
1979); or Tschemper et al., Gene 10:157-166, 1980). The
trp1 gene provides a selection marker for a mutant strain

15   of yeast lacking the ability to grow in tryptophan, for
example, ATCC No. 44076. The presence of the trp1 lesion
in the yeast host cell genome then provides an effective
environment for detecting transformation by growth in the
absence of tryptophan. Similarly, Leu2-deficient yeast

20   strains (ATCC 20,622 or 38,626) are complemented by known
plasmids bearing the Leu2 gene.

                    e.   Promoter Component
               Expression vectors usually contain a promoter that is

25   recognized by the host organism and is operably linked to
C-C CKR-1 nucleic acid. Promoters are untranslated
sequences located upstream (5') to the start codon of a
structural gene (generally within about 100 to 1000 bp)
that control the transcription and translation of a

30   particular nucleic acid sequence, such as C-C CKR-1, to
which they are operably linked. Such promoters typically
fall into two classes, inducible and constitutive.
Inducible promoters are promoters that initiate increased
levels of transcription from DNA under their control in

35   response to some change in culture conditions, e.g. the
presence or absence of a nutrient or a change in
temperature. At this time a large number of promoters
recognized by a variety of potential host cells are well
known. These promoters are operably linked to DNA encoding

21

C-C CKR-1 by removing the promoter from the source DNA by
restriction enzyme digestion and inserting the isolated
promoter sequence into the vector. Both the native C-C
CKR-1 promoter sequence and many heterologous promoters may

5   be used to direct amplification and/or expression of C-C
CKR-1 DNA. However, heterologous promoters are preferred,
as they generally permit greater transcription and higher
yields of expressed C-C CKR-1 as compared to the native C-C
CKR-1 promoter.

10   Promoters suitable for use with prokaryotic hosts
include the ß-lactamase and lactose promoter systems (Chang
et al., Nature 275:617-624, 1978); and Goeddel et al.,
Nature 281:544-548, 1979), alkaline phosphatase, a
tryptophan (trp) promoter system (Goeddel, Nucleic Acids

15   Res. 8(18):4057-4074, 1980) and EP 36,776) and hybrid
promoters such as the tac promoter (deBoer et al., Proc.
Natl. Acad. Sci. U.S.A. 80:21-25, 1983). However, other
known bacterial promoters are suitable. Their nucleotide
sequences have been published, thereby enabling a skilled

20   worker to operably ligate them to DNA encoding C-C CKR-1
(Siebenlist et al., Cell 20:269-281, 1980) using linkers or
adapters to supply any required restriction sites.
Promoters for use in bacterial systems also generally will
contain a Shine-Dalgarno (S.D.) sequence operably linked to

25   the DNA encoding C-C CKR-1.
       Suitable promoting sequences for use with yeast hosts
include the promoters for 3-phosphoglycerate kinase
(Hitzeman et al., J. Biol. Chem. 255(24):12073-80, 1980) or
other glycolytic enzymes (Hess et al., J. Adv. Enzyme Reg.

.30 7:149-67, 1968); and Holland, Biochemistry 17:4900-4907,
1978), such as enolase, glyceraldehyde-3-phosphate
dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-
fructokinase, glucose-6-phosphate isomerase, 3-
phosphoglycerate mutase, pyruvate kinase, triosephosphate

35   isomerase, phosphoglucose isomerase, and glucokinase.
       Other yeast promoters, which are inducible promoters
having the additional advantage of transcription controlled
by growth conditions, are the promoter regions for alcohol
dehydrogenase 2, isocytochrome C, acid phosphatase,

22

degradative enzymes associated with nitrogen metabolism,
metallothionein, glyceraldehyde-3-phosphate dehydrogenase,
and enzymes responsible for maltose and galactose
utilization. Suitable vectors and promoters for use in
5   yeast expression are further described in Hitzeman et al.,
EP 73,657A. Yeast enhancers also are advantageously used
with yeast promoters.

Promoter sequences are known for eukaryotes.
·Virtually all eukaryotic genes have an AT-rich region
10 located approximately 25 to 30 bases upstream from the site
where transcription is initiated. Another sequence found
70 to 80 bases upstream from the start of transcription of
many genes is a CXCAAT region where X may be any
nucleotide. At the 3' end of most eukaryotic genes is an
15 AATAAA sequence that may be the signal for addition of the
poly A tail to the 3' end of the coding sequence. All of
these sequences are suitably inserted into mammalian
expression vectors.

C-C CKR-1 transcription from vectors in mammalian host
20 cells is controlled by promoters obtained from the genomes
of viruses such as polyoma virus, fowlpox virus (UK
2,211,504 published 5 July 1989), adenovirus (such as
Adenovirus 2), bovine papilloma virus, avian sarcoma virus,
cytomegalovirus, a retrovirus, hepatitis B virus and most
25 preferably Simian Virus 40 (SV40), from heterologous
mammalian promoters, e.g. the actin promoter or an
immunoglobulin promoter, from heat-shock promoters, and
from the promoter normally associated with C-C CKR-1
sequence, provided such promoters are compatible with the
30 host cell systems.   :

The early and late promoters of the SV40 virus are
conveniently obtained as an SV40 restriction fragment that
also contains the SV40 viral origin of replication (Fiers
et al., _Nature_ 273:113-120, 1978; Mulligan and Berg,
35 _Science_ 209:1422-1427, 1980; Pavlakis et al., _Proc. Natl._
Acad. Sci. U.S.A. 78:7398-7402, 1981). The immediate early
promoter of the human cytomegalovirus is conveniently
obtained as a HindIII E restriction fragment (Greenaway et
al., _Gene_ 18:355-360, 1982). A system for expressing DNA in

23

mammalian hosts using the bovine papilloma virus as a
vector is disclosed in U.S. 4,419,446. A modification of
this system is described in U.S. 4,601,978. See also Gray
et al., _Nature_ 295:503-508, 1982, on expressing cDNA

5   encoding immune interferon in monkey cells; Reyes et al.,
Nature 297:598-601, 1982, on expression of human ß-
interferon cDNA in mouse cells under the control of a
thymidine kinase promoter from herpes simplex virus,
Canaani and Berg, _Proc. Natl. Acad. Sci. U.S.A._ 79:5166-

10   5170, 1982, on expression of the human interferon ß1 gene
in cultured mouse and rabbit cells, and Gorman et al.,
Proc. Natl. Acad. Sci. U.S.A. 79:6777-6781, 1982, on
expression of bacterial CAT sequences in CV-1 monkey kidney
cells, chicken embryo fibroblasts, Chinese hamster ovary

15   cells, HeLa cells, and mouse NIH-3T3 cells using the Rous
sarcoma virus long terminal repeat as a promoter.

                f.   _Enhancer Element Component_
        Transcription of a DNA encoding C-C CKR-1 of this
20   invention by higher eukaryotes is often increased by
inserting an enhancer sequence into the vector. Enhancers
are cis-acting elements of DNA, usually about from 10-300
bp, that act on a promoter to increase its transcription.
Enhancers are relatively orientation and position

25   independent having been found 5' (Laimins et al., _Proc._
Natl. Acad. Sci. U.S.A. 78:464-8, 1981) and 3' (Lusky et
al., _Mol. Cell Bio._ 3(6):1108-1122, 1983) to the
transcription unit, within an intron (Banerji et al., _Cell_
33:729-740, 1983) as well as within the coding sequence

30   itself (Osborne et al., _Mol. Cell Bio._ 4(7):1293-1305,
1984). Many enhancer sequences are now known from
mammalian genes (globin, elastase, albumin, α-fetoprotein
and insulin). Typically, however, one will use an enhancer
from a eukaryotic cell virus. Examples include the SV40

35   enhancer on the late side of the replication origin (bp
100-270), the cytomegalovirus early promoter enhancer, the
polyoma enhancer on the late side of the replication
origin, and adenovirus enhancers. See also Yaniv, _Nature_
297:17-18, 1982, on enhancing elements for activation of

24

eukaryotic promoters. The enhancer may be spliced into the
vector at a position 5' or 3' to C-C CKR-1 DNA, but is
preferably located at a site 5' from the promoter.

5   g. Transcription Termination Component
        Expression vectors used in eukaryotic host cells
(yeast, fungi, insect, plant, animal, human, or nucleated
cells from other multicellular organisms) will also contain
sequences necessary for the termination of transcription
10   and for stabilizing the mRNA. Such sequences are commonly
available from the 5' and, occasionally 3' untranslated
regions of eukaryotic or viral DNAs or cDNAs. These
regions contain nucleotide segments transcribed as
polyadenylated fragments in the untranslated portion of the
15   mRNA encoding C-C CKR-1. The 3' untranslated regions also
include transcription termination sites.
        Suitable vectors containing one or more of the above
listed components and the desired coding and control
sequences are constructed by standard ligation techniques.
20   Isolated plasmids or DNA fragments are cleaved, tailored,
and religated in the form desired to generate the plasmids
required.
        For analysis to confirm correct sequences in plasmids
constructed, the ligation mixtures are used to transform E.
25   coli K12 strain 294 (ATCC 31,446) and successful
transformants selected by ampicillin or tetracycline
resistance where appropriate. Plasmids from the
transformants are prepared, analyzed by restriction
endonuclease digestion, and/or sequenced by the method of
30   Messing et al., Nucleic Acids Res. 9(2):309-321, 1981, or
by the method of Maxam et al., Methods in Enzymology
65:499-560, 1980.
        Particularly useful in the practice of this invention
are expression vectors that provide for the transient
35   expression in mammalian cells of DNA encoding C-C CKR-1.
In general, transient expression involves the use of an
expression vector that is able to replicate efficiently in
a host cell, such that the host cell accumulates many
copies of the expression vector and, in turn, synthesizes

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high levels of a desired polypeptide encoded by the
expression vector. Transient expression systems,
comprising a suitable expression vector and a host cell,
allow for the convenient positive identification of
5   polypeptides encoded by cloned DNAs, as well as for the
rapid screening of such polypeptides for desired biological
or physiological properties. Thus, transient expression
systems are particularly useful in the invention for
purposes of identifying analogs and variants of C-C CKR-1
10   that have C-C CKR-1-like activity, and for analysis of the
effect of the binding of chemokine variants to C-C CKR-1.

Other methods, vectors, and host cells suitable for
adaptation to the synthesis of C-C CKR-1 in recombinant
vertebrate cell culture are described in Gething et al.,
15   Nature 293:620-625, 1981; Mantei et al., Nature 281:40-46,
1979); EP 117,060; and EP 117,058. A particularly useful
plasmid for mammalian cell culture expression of C-C CKR-1
is pRK5 (EP pub. no. 307,247) or pSVI6B (U.S. Ser. No.
07/441,574 filed 22 November 1989).

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4. Selection and Transformation of Host Cells
Suitable host cells for cloning or expressing C-C CKR-
1 expression vectors are the prokaryote, yeast, or higher
eukaryotic cells described above. Suitable prokaryotes
25   include eubacteria, such as Gram-negative or Gram-positive
organisms, for example, E. coli, Bacilli such as B.
subtilis, Pseudomonas species such as P. aeruginosa,
Salmonella typhimurium, or Serratia marcescens. One
preferred E. coli cloning host is E. coli 294 (ATCC
30   31,446), although other strains such as E. coli B, E. coli
χ1776 (ATCC 31,537), and E. coli W3110 (ATCC 27,325) are
suitable. These examples are illustrative rather than
limiting. Preferably the host cell should secrete minimal
amounts of proteolytic enzymes. Alternatively, in vitro
35   methods of cloning, e.g. PCR or other nucleic acid
polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such
as filamentous fungi or yeast are suitable hosts for
vectors containing C-C CKR-1 DNA. Saccharomyces

cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful to practice the invention, such as S.

5   pombe (Beach and Nurse, Nature 290:140-143, 1981), Kluyveromyces lactis (Louvencourt et al., J. Bacteriol. 154(2):737-742, 1983), Pichia pastoris (EP 183,070), Trichoderma reesia (EP 244,234), Neurospora crassa (Case et al., Proc. Natl. Acad. Sci. U.S.A. 76:5259-5263, 1979), and

10   Aspergillus hosts such as A. nidulans (Ballance et al., Biochem. Biophys. Res. Commun. 112:284-289, 1983); Tilburn et al., Gene 26:205-221, 1983); Yelton et al., Proc. Natl. Acad. Sci. U.S.A. 81:1470-1474, 1984) and A. niger (Kelly and Hynes, EMBO J. 4:475-479, 1985).

15   Suitable host cells for the expression of glycosylated C-C CKR-1 polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from

20   vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera frugiperda (caterpillar), Aedes aegypti (mosquito), Aedes

25   albopictus (mosquito), Drosophila melanogaster (fruit fly), and Bombyx mori host cells have been identified. See, e.g., Luckow et al., Bio/Technology 6:47-55, 1988); Miller et al., in Genetic Engineering, Setlow, J.K. et al., 8:277-279 (Plenum Publishing, 1986), and Maeda et al., Nature

30   315:592-594, 1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV, and such viruses may be used as the virus according to the present invention, particularly for transfection of

35   Spodoptera frugiperda cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium Agrobacterium tumefaciens,

which has been previously manipulated to contain C-C CKR-1
DNA. During incubation of the plant cell culture with A.
tumefaciens, the DNA encoding C-C CKR-1 is transferred to
the plant cell host such that it is transfected, and will,
5   under appropriate conditions, express C-C CKR-1 DNA. In
addition, regulatory and signal sequences compatible with
plant cells are available, such as the nopaline synthase
promoter and polyadenylation signal sequences. Depicker et
al., <u>J. Mol. Appl. Gen.</u> 1: 561-573, 1982). In addition,
10 DNA segments isolated from the upstream region of the T-DNA
780 gene are capable of activating or increasing
transcription levels of plant-expressible genes in
recombinant DNA-containing plant tissue. See EP 321,196
published 21 June 1989.

15   However, interest has been greatest in vertebrate
cells, and propagation of vertebrate cells in culture
(tissue culture) has become a routine procedure in recent
years (<u>Tissue Culture</u>, Academic Press, Kruse and Patterson,
eds., 1973). Examples of useful mammalian host cell lines
20 are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC
CRL 1651); human embryonic kidney line (293 or 293 cells
subcloned for growth in suspension culture, Graham et al.,
<u>J. Gen. Virol.</u> 36:59-72, 1977); baby hamster kidney cells
(BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO,
25 Urlaub and Chasin, <u>Proc. Natl. Acad. Sci. U.S.A.</u>
77(7):4216-4220, 1980); mouse sertoli cells (TM4, Mather,
<u>Biol. Reprod.</u> 23:243-251, 1980); monkey kidney cells (CV1
ATCC CCL 70); African green monkey kidney cells (VERO-76,
ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC
30 CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo
rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells
(W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065);
mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells
(Mather et al., <u>Annals N.Y. Acad. Sci.</u> 383:44-68, 1982);
35 MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep
G2). Preferred host cells are human embryonic kidney 293
and Chinese hamster ovary cells.

The host chosen for expression may also be a
multicellular organism, as in a transgenic animal. Such

animals have been produced by transfection of germ cells,
somatic cells, or embryos with heterologous DNA, suitably
implanting the transfected cells and allowing the cells to
mature into or stably integrate into adult animals

5   containing the heterologous DNA. A reproducible percentage
of such animals transcribe and express the heterologous DNA
as protein which can be identified in tissues including
blood or serum. Suitable methods for making transgenic
animals are described in U.S. Patent 4,396,601 and Palmiter

10 et al., Nature 300:611-615, 1982.

Host cells are transfected and preferably transformed
with the above-described expression or cloning vectors of
this invention and cultured in conventional nutrient media
modified as appropriate for inducing promoters, selecting

15 transformants, or amplifying the genes encoding the desired
sequences.

Transfection refers to the taking up of an expression
vector by a host cell whether or not any coding sequences
are in fact expressed. Numerous methods of transfection

20 are known to the ordinarily skilled artisan, for example,
$CaPO_4$ and electroporation. Successful transfection is
generally recognized when any indication of the operation
of this vector occurs within the host cell.

Transformation means introducing DNA into an organism

25 so that the DNA is replicable, either as an
extrachromosomal element or by chromosomal integrant.
Depending on the host cell used, transformation is done
using standard techniques appropriate to such cells. The
calcium treatment employing calcium chloride, as described

30 in section 1.82 of Sambrook et al., is generally used for
prokaryotes or other cells that contain substantial cell-
wall barriers. Infection with Agrobacterium tumefaciens is
used for transformation of certain plant cells, as
described by Shaw et al., Gene 23:315-330, 1983) and WO

35 89/05859, published 29 June 1989. For mammalian cells
without such cell walls, the calcium phosphate
precipitation method described in sections 16.30-16.37 of
Sambrook et al., supra, is preferred. General aspects of
mammalian cell host system transformations have been

described by Axel in U.S. 4,399,216, issued 16 August 1983.
Transformations into yeast are typically carried out
according to the method of Van Solingen et al., _J._
Bacteriol. 130(2):946-947, 1977) and Hsiao et al., _Proc._
5   _Natl. Acad. Sci. U.S.A._, 76(8):3829-3833, 1979). However,
other methods for introducing DNA into cells such as by
nuclear injection, electroporation, or by protoplast fusion
may also be used.

10   5. _Culturing the Host Cells_

    Prokaryotic cells used to produce C-C CKR-1
polypeptide of this invention are cultured in suitable
media as described generally in Sambrook et al., supra.
15   The mammalian host cells used to produce C-C CKR-1
of this invention may be cultured in a variety of media.
Commercially available media such as Ham's F10 (Sigma),
Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma),
and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are
20   suitable for culturing the host cells. In addition, any of
the media described in Ham and McKeehan, _Meth. Enz._ 58:44-
93, 1979, Barnes and Sato, _Anal. Biochem._ 102:255-270,
1980, U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655;
WO 90/03430; WO 87/00195; U.S. Pat. Re. 30,985; or U.S.
25   5,122,469 may be used as culture media for the host cells.
Any of these media may be supplemented as necessary with
hormones and/or other growth factors (such as insulin,
transferrin, or epidermal growth factor), salts (such as
sodium chloride, calcium, magnesium, and phosphate),
30   buffers (such as HEPES), nucleosides (such as adenosine and
thymidine), antibiotics (such as Gentamycin^{TM} drug), trace
elements (defined as inorganic compounds usually present at
final concentrations in the micromolar range), and glucose
or an equivalent energy source. Any other necessary
35   supplements may also be included at appropriate
concentrations that would be known to those skilled in the
art. The culture conditions, such as temperature, pH, and
the like, are those previously used with the host cell
selected for expression, and will be apparent to the

ordinarily skilled artisan.

The host cells referred to in this disclosure
encompass cells in in vitro culture as well as cells that
are within a host animal.

5

6. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured
in a sample directly, for example, by conventional Southern
blotting, northern blotting to quantitate the transcription
10 of mRNA (Thomas, *Proc. Natl. Acad. Sci. U.S.A.*
77:5201-5205, 1980), dot blotting (DNA analysis), or in
situ hybridization, using an appropriately labeled probe.
Various labels may be employed, most commonly
radioisotopes, particularly ^{32}P. However, other techniques
15 may also be employed, such as using biotin-modified
nucleotides for introduction into a polynucleotide. The
biotin then serves as the site for binding to avidin or
antibodies, which may be labeled with a wide variety of
labels, such as radionuclides, fluorescers, enzymes, or the
20 like.

Alternatively, antibodies may be employed that can
recognize specific duplexes, including DNA duplexes, RNA
duplexes, and DNA-RNA hybrid duplexes or DNA-protein
duplexes. The antibodies in turn may be labeled and the
25 assay may be carried out where the duplex is bound to a
surface, so that upon the formation of duplex on the
surface, the presence of antibody bound to the duplex can
be detected.

Gene expression, alternatively, may be measured by
30 immunological methods, such as immunohistochemical staining
of tissue sections and assay of cell culture or body
fluids, to quantitate directly the expression of gene
product. With immunohistochemical staining techniques, a
cell sample is prepared, typically by dehydration and
35 fixation, followed by reaction with labeled antibodies
specific for the gene product coupled, where the labels are
usually visually detectable, such as enzymatic labels,
fluorescent labels, luminescent labels, and the like. A
particularly sensitive staining technique suitable for use

in the present invention is described by Hsu et al.,
Am. J. Clin. Path. 75:734-738, 1980.

Antibodies useful for immunohistochemical staining
and/or assay of sample fluids may be either monoclonal or
5   polyclonal, and may be prepared in any mammal.
Conveniently, the antibodies may be prepared against a
native or synthetic C-C CKR-1 polypeptide or variant
thereof.

10   7.   Purification of C-C CKR-1 Polypeptide
C-C CKR-1 is recovered from cell cultures by
solubilizing cell membranes in detergent.

When a human C-C CKR-1 is expressed in a recombinant
cell other than one of human origin, C-C CKR-1 is
15   completely free of proteins or polypeptides of human
origin. However, it is necessary to purify C-C CKR-1 from
recombinant cell proteins or polypeptides to obtain
preparations that are substantially homogeneous by protein
as to C-C CKR-1. As a first step, the cells are
20   centrifuged to separate them from culture medium, followed
by suitable purification procedures such as: fractionation
on immunoaffinity or ion-exchange columns; ethanol
precipitation; reverse phase HPLC; chromatography on silica
or on a cation exchange resin such as DEAE;
25   chromatofocusing; SDS-PAGE; ammonium sulfate precipitation;
gel filtration using, for example, Sephadex G-75.

C-C CKR-1 variants in which residues have been
deleted, inserted or substituted are recovered in the same
fashion as the native C-C CKR-1, taking account of any
30   substantial changes in properties occasioned by the
variation. For example, preparation of a C-C CKR-1 fusion
with another protein or polypeptide, e.g. a bacterial or
viral antigen, facilitates purification; an immunoaffinity
column containing antibody to the antigen can be used to
35   adsorb the fusion. Immunoaffinity columns such as a rabbit
polyclonal anti-C-C CKR-1 column can be employed to absorb
C-C CKR-1 variant by binding it to at least one remaining
immune epitope. A protease inhibitor such as phenyl methyl
sulfonyl fluoride (PMSF) also may be useful to inhibit

32

proteolytic degradation during purification, and
antibiotics may be included to prevent the growth of
adventitious contaminants. One skilled in the art will
appreciate that purification methods suitable for native C-
5   C CKR-1 may require modification to account for changes in
the character of C-C CKR-1 or its variants upon expression
in recombinant cell culture.

        8. Covalent Modifications of C-C CKR-1 Polypeptides
10   Covalent modifications of C-C CKR-1 polypeptide or its
glycosyl substituents are included within the scope of this
invention. Both native C-C CKR-1 and amino acid sequence
variants of C-C CKR-1 may be covalently modified. Covalent
modifications of C-C CKR-1, fragments thereof or antibodies
15   thereto are introduced into the molecule by reacting
targeted amino acid residues of C-C CKR-1, fragments
thereof, or C-C CKR-1 antibody with an organic derivatizing
agent that is capable of reacting with selected side chains
or the N- or C-terminal residues. Most commonly, C-C CKR-1
20   and its antibodies are covalently bonded to detectable
groups used in diagnosis, e.g. enzymes, radio isotopes,
spin labels, antigens, fluorescent or chemiluminescent
groups and the like.
        Cysteinyl residues most commonly are reacted with α-
25   haloacetates (and corresponding amines), such as
chloroacetic acid or chloroacetamide, to give carboxymethyl
or carboxyamidomethyl derivatives. Cysteinyl residues also
are derivatized by reaction with bromotrifluoroacetone, α-
bromo-ß-(5-imidazole)propionic acid, chloroacetyl
30   phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide,
methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-
chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-
1,3-diazole.
        Histidyl residues are derivatized by reaction with
35   diethylpyrocarbonate at pH 5.5-7.0 because this agent is
relatively specific for the histidyl side chain. Para-
bromophenacyl bromide also is useful; the reaction is
preferably performed in 0.1 M sodium cacodylate at pH 6.0.
        Lysinyl and amino terminal residues are reacted with

succinic or other carboxylic acid anhydrides.
Derivatization with these agents has the effect of
reversing the charge of the lysinyl residues. Other
suitable reagents for derivatizing α-amino-containing
5   residues include imidoesters such as methyl picolinimidate;
pyridoxal phosphate; pyridoxal; chloroborohydride;
trinitrobenzenesulfonic acid; O-methylisourea; 2,4-
pentanedione; and transaminase-catalyzed reaction with
glyoxylate.
10   Arginyl residues are modified by reaction with one or
several conventional reagents, among them phenylglyoxal,
2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin.
Derivatization of arginine residues requires that the
reaction be performed in alkaline conditions because of the
15 high pK_a of the guanidine functional group. Furthermore,
these reagents may react with the groups of lysine as well
as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be
made, with particular interest in introducing spectral
20 labels into tyrosyl residues by reaction with aromatic
diazonium compounds or tetranitromethane. Most commonly,
N-acetylimidizole and tetranitromethane are used to form O-
acetyl tyrosyl species and 3-nitro derivatives,
respectively. Tyrosyl residues are iodinated using ^{125}I or
25 ^{131}I to prepare labeled proteins for use in
radioimmunoassay, the chloramine T method described above
being suitable.

Carboxyl side groups (aspartyl or glutamyl) are
selectively modified by reaction with carbodiimides (R'-
30 N=C=N-R'), where R and R' are different alkyl groups, such
as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or
1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide.
Furthermore, aspartyl and glutamyl residues are converted
to asparaginyl and glutaminyl residues by reaction with
35 ammonium ions.

Derivatization with bifunctional agents is useful for
cross-linking C-C CKR-1, its fragments or antibodies to a
water-insoluble support matrix or surface for use in
methods for purifying anti-C-C CKR-1 antibodies, and vice

34

versa.   Commonly used cross-linking agents include, e.g.,
1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-
hydroxysuccinimide esters, for example, esters with 4-
azidosalicylic acid, homobifunctional imidoesters,
5   including disuccinimidyl esters such as 3,3'-dithiobis-
(succinimidylpropionate), and bifunctional maleimides such
as bis-N-maleimido-1,8-octane.   Derivatizing agents such as
methyl-3-[(p-azidophenyl)dithio]propioimidate yield
photoactivatable intermediates that are capable of forming
10 cross-links in the presence of light.   Alternatively,
reactive water-insoluble matrices such as cyanogen bromide-
activated carbohydrates and the reactive substrates
described in U.S. 3,969,287; 3,691,016; 4,195,128;
4,247,642; 4,229,537; and 4,330,440 are employed for
15 protein immobilization.

     Glutaminyl and asparaginyl residues are frequently
deamidated to the corresponding glutamyl and aspartyl
residues, respectively.   Alternatively, these residues are
deamidated under mildly acidic conditions.   Either form of
20 these residues falls within the scope of this invention.

     Other modifications include hydroxylation of proline
and lysine, phosphorylation of hydroxyl groups of seryl or
threonyl residues, methylation of the α-amino groups of
lysine, arginine, and histidine side chains (T.E.
25 Creighton, Proteins: Structure and Molecular Properties,
W.H. Freeman & Co., San Francisco, pp. 79-86 1983),
acetylation of the N-terminal amine, and amidation of any
C-terminal carboxyl group.

     Another type of covalent modification of C-C CKR-1
30 polypeptide included within the scope of this invention
comprises altering the native glycosylation pattern of the
polypeptide.   By altering is meant deleting one or more
carbohydrate moieties found in the native polypeptide,
and/or adding one or more glycosylation sites that are not
35 present in the native polypeptide.

     Glycosylation of polypeptides is typically either N-
linked or O-linked.   N-linked refers to the attachment of
the carbohydrate moiety to the side chain of an asparagine
residue.   The tri-peptide sequences asparagine-X-serine and

asparagine-X-threonine, where X is any amino acid except
proline, are the recognition sequences for enzymatic
attachment of the carbohydrate moiety to the asparagine
side chain. Thus, the presence of either of these tri-
5   peptide sequences in a polypeptide creates a potential
glycosylation site. O-linked glycosylation refers to the
attachment of one of the sugars N-acetylgalactosamine,
galactose, or xylose, to a hydroxyamino acid, most commonly
serine or threonine, although 5-hydroxyproline or 5-
10   hydroxylysine may also be used.

Addition of glycosylation sites to C-C CKR-1
polypeptide is conveniently accomplished by altering the
amino acid sequence such that it contains one or more of
the above-described tri-peptide sequences (for N-linked
15   glycosylation sites). The alteration may also be made by
the addition of, or substitution by, one or more serine or
threonine residues to the native C-C CKR-1 sequence (for O-
linked glycosylation sites). For ease, C-C CKR-1 amino
acid sequence is preferably altered through changes at the
20   DNA level, particularly by mutating the DNA encoding C-C
CKR-1 polypeptide at preselected bases such that codons are
generated that will translate into the desired amino acids.
The DNA mutation(s) may be made using methods described
above under the heading of "Amino Acid Sequence Variants of
25   C-C CKR-1 Polypeptide".

Another means of increasing the number of carbohydrate
moieties on C-C CKR-1 polypeptide is by chemical or
enzymatic coupling of glycosides to the polypeptide. These
procedures are advantageous in that they do not require
30   production of the polypeptide in a host cell that has
glycosylation capabilities for N- and O- linked
glycosylation. Depending on the coupling mode used, the
sugar(s) may be attached to (a) arginine and histidine, (b)
free carboxyl groups, (c) free sulfhydryl groups such as
35   those of cysteine, (d) free hydroxyl groups such as those
of serine, threonine, or hydroxyproline, (e) aromatic
residues such as those of phenylalanine, tyrosine, or
tryptophan, or (f) the amide group of glutamine. These
methods are described in WO 87/05330 published 11 September

36

1987, and in Aplin and Wriston (CRC Crit. Rev. Biochem. pp. 259-306, 1981).

Removal of carbohydrate moieties present on the native C-C CKR-1 polypeptide may be accomplished chemically or
5   enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while
10   leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al. (Arch. Biochem. Biophys. 259:52-57, 1987) and by Edge et al. (Anal. Biochem. 118:131-137, 1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a
15   variety of endo- and exo- glycosidases as described by Thotakura et al. (Meth. Enzymol. 138:350-359, 1987).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al. (J. Biol. Chem. 257:3105-3109,
20   1982). Tunicamycin blocks the formation of protein-N-glycoside linkages.

The C-C CKR-1 also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example,
25   hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are
30   disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., ed., 1980).

C-C CKR-1 preparations are also useful in generating antibodies, for use as standards in assays for C-C CKR-1 (e.g. by labeling C-C CKR-1 for use as a standard in a
35   radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

37

Since it is often difficult to predict in advance the characteristics of a variant C-C CKR-1, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example,

5   a change in the immunological character of C-C CKR-1 molecule, such as affinity for a given antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity observed for

10   native C-C CKR-1 in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity; susceptibility to proteolytic degradation, or the tendency to aggregate with carriers or into multimers are assayed by methods well

15   known in the art.

        9. Therapeutic Compositions and Administration of C-C
CKR-1

        Therapeutic formulations of C-C CKR-1 (including its

20   C-C CKR-1 binding fragments) or antibodies thereto are prepared for storage by mixing C-C CKR-1 having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra), in the form of lyophilized

25   cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than

30   about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose,

35   mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics, or polyethylene glycol (PEG).

        The C-C CKR-1 or antibody to be used for in vivo

38

administration must be sterile. This is readily
accomplished by filtration through sterile filtration
membranes, prior to or following lyophilization and
reconstitution. The C-C CKR-1 ordinarily will be stored in
5 lyophilized form or in solution.

Therapeutic C-C CKR-1 or antibody compositions
generally are placed into a container having a sterile
access port, for example, an intravenous solution bag or
vial having a stopper pierceable by a hypodermic injection
10 needle.

The route of C-C CKR-1 or antibody administration is
in accord with known methods, e.g. injection or infusion by
intravenous, intraperitoneal, intracerebral, intramuscular,
intraocular, intraarterial, or intralesional routes, or by
15 sustained release systems as noted below.

Suitable examples of sustained-release preparations
include semipermeable polymer matrices in the form of
shaped articles, e.g. films, or microcapsules. Sustained
release matrices include polyesters, hydrogels,
20 polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-
glutamic acid and γ ethyl-L-glutamate (Sidman et al.,
Biopolymers 22:547-556, 1983), poly (2-hydroxyethyl-
methacrylate) (Langer et al., J. Biomed. Mater. Res.
15:167-277, 1981; Langer, Chem. Tech., 12:98-105, 1982),
25 ethylene vinyl acetate (Langer et al., supra) or poly-D-(-
)-3-hydroxybutyric acid (EP 133,988). Sustained-release C-
C CKR-1 or antibody compositions also include liposomally
entrapped C-C CKR-1 or antibody. Liposomes containing C-C
CKR-1 or antibody are prepared by methods known per se: DE
30 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. U.S.A.
82:3688-3692, 1985; Hwang et al., Proc. Natl. Acad. Sci.
U.S.A. 77:4030-4034, 1980; EP 52,322; EP 36,676; EP 88,046;
EP 143,949; EP 142,641; Japanese patent application 83-
118008; U.S. 4,485,045 and 4,544,545; and EP 102,324.
35 Ordinarily the liposomes are of the small (about 200-800
Angstroms) unilamelar type in which the lipid content is
greater than about 30 mol. % cholesterol, the selected
proportion being adjusted for the optimal C-C CKR-1 or
antibody therapy.

39

An effective amount of C-C CKR-1 or antibody to be
employed therapeutically will depend, for example, upon the
therapeutic objectives, the route of administration, and
the condition of the patient. For example, it is expected
5   that C-C CKR-1 will be therapeutically effective in the
treatment of cytokine-mediated inflammation. Accordingly,
it will be necessary for the therapist to titer the dosage
and modify the route of administration as required to
obtain the optimal therapeutic effect. Typically, the
10   clinician will administer C-C CKR-1 or antibody until a
dosage is reached that achieves the desired effect. The
progress of this therapy is easily monitored by
conventional assays.

15   10. C-C CKR-1 Antibody Preparation
Polyclonal antibodies to C-C CKR-1 generally are
raised in animals by multiple subcutaneous (sc) or
intraperitoneal (ip) injections of C-C CKR-1 and an
adjuvant. Immunization with recombinant cells transformed
20   with C-C CKR-1 (e.g. mouse or CHO cells transformed with
human C-C CKR-1) may be satisfactory, or it may be useful
to separate C-C CKR-1 and conjugate it or a fragment
containing the target amino acid sequence to a protein that
is immunogenic in the species to be immunized, e.g.,
25   keyhole limpet hemocyanin, serum albumin, bovine
thyroglobulin, or soybean trypsin inhibitor using a
bifunctional or derivatizing agent, for example,
maleimidobenzoyl sulfosuccinimide ester (conjugation
through cysteine residues), N-hydroxysuccinimide (through
30   lysine residues), glutaraldehyde, succinic anhydride,
$SOCl_2$, or $R^1N = C = NR$, where R and R^1 are different alkyl
groups.
Animals ordinarily are immunized against the cells or
immunogenic conjugates or derivatives by combining 1 mg or
35   1 µg of C-C CKR-1 in Freund's complete adjuvant and
injecting the solution intradermally at multiple sites.
One month later the animals are boosted with 1/5 to 1/10
the original amount of conjugate in Freund's complete
adjuvant by subcutaneous injection at multiple sites. 7 to

14 days later animals are bled and the serum is assayed for
anti-C-C CKR-1 titer. Animals are boosted until the titer
plateaus. Preferably, the animal is boosted with the
conjugate of the same C-C CKR-1, but conjugated to a

5   different protein and/or through a different cross-linking
agent. Conjugates also can be made in recombinant cell
culture as protein fusions. Also, aggregating agents such
as alum are used to enhance the immune response.

      Another option is to employ combinatorial variable

10   domain libraries and screening methods to identify the
desired anti-C-C CKR-1 antibodies.

      Monoclonal antibodies are prepared by recovering
spleen cells from immunized animals and immortalizing the
cells in conventional fashion, e.g. by fusion with myeloma

15   cells or by Epstein-Barr (EB)-virus transformation and
screening for clones expressing the desired antibody.

      The monoclonal antibody preferably is specific for
each target C-C CKR-1 polypeptide. The antibody is
selected to be either agonistic, antagonistic or to have no

20   effect on the activity of or binding of the C-C CKR-1.

      11. Uses of C-C CKR-1 Nucleic Acid, and Antibodies

      The nucleic acid encoding C-C CKR-1 may be used as a
diagnostic for tissue specific typing. For example, such

25   procedures as in situ hybridization, and northern and
Southern blotting, and PCR analysis may be used to
determine whether DNA and/or RNA encoding C-C CKR-1 are
present in the cell type(s) being evaluated.

      Isolated C-C CKR-1 polypeptide may be used in

30   quantitative diagnostic assays as a standard or control
against which samples e.g., from erythrocytes, containing
unknown quantities of C-C CKR-1 may be compared.
Recombinant cells which express C-C CKR-1 can be used in
assays for C-C CKR-1 ligands in the same fashion as for

35   example neutrophils are used in IL-8 assays. The C-C CKR-1
polypeptides, fragments or cells (as such, or derivatized)
also can be used as immunogens in the production of
antibodies to C-C CKR-1, or for the purification of such
antibodies from ascites or recombinant cell culture media.

41

C-C CKR-1 antibodies are useful in diagnostic assays
for C-C CKR-1 expression in specific cells or tissues
wherein the antibodies are labeled in the same fashion as
C-C CKR-1 described above and/or are immobilized on an
5   insoluble matrix. C-C CKR-1 antibodies also are useful for
the affinity purification of C-C CKR-1 from recombinant
cell culture or natural sources. The C-C CKR-1 antibodies
that do not detectably cross-react with other chemokine
receptors can be used to purify each C-C CKR-1 free from
10 other homologous chemokine receptors. C-C CKR-1 antibodies
that are PF4 superfamily antagonists are useful as anti-
inflammatory agents or in the treatment of other PF4
superfamily-mediated disorders.

Suitable diagnostic assays for C-C CKR-1 and its
15 antibodies are well known per se. Such assays include
competitive and sandwich assays, and steric inhibition
assays. Competitive and sandwich methods employ a phase-
separation step as an integral part of the method while
steric inhibition assays are conducted in a single reaction
20 mixture. Fundamentally, the same procedures are used for
the assay of C-C CKR-1 and for substances that bind C-C
CKR-1, although certain methods will be favored depending
upon the molecular weight of the substance being assayed.
Therefore, the substance to be tested is referred to as an
25 analyte, irrespective of its status otherwise as an antigen
or antibody, and proteins that bind to the analyte are
denominated binding partners, whether they be antibodies,
cell surface receptors, or antigens.

Analytical methods for C-C CKR-1 or its antibodies all
30 use one or more of the following reagents: labeled analyte
analog, immobilized analyte analog, labeled binding
partner, immobilized binding partner and steric conjugates.
The labeled reagents also are known as "tracers."

The label used (and this is also useful to label C-C
35 CKR-1 nucleic acid for use as a probe) is any detectable
functionality that does not interfere with the binding of
analyte and its binding partner. Numerous labels are known
for use in immunoassay, examples including moieties that
may be detected directly, such as fluorochrome,

42

chemiluminescent, and radioactive labels, as well as
moieties, such as enzymes, that must be reacted or
derivatized to be detected. Examples of such labels
include the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I,

5   fluorophores such as rare earth chelates or fluorescein and
its derivatives, rhodamine and its derivatives, dansyl,
umbelliferone, luceriferases, e.g., firefly luciferase and
bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin,
2,3-dihydrophthalazinediones, horseradish peroxidase (HRP),

10   alkaline phosphatase, ß-galactosidase, glucoamylase,
lysozyme, saccharide oxidases, e.g., glucose oxidase,
galactose oxidase, and glucose-6-phosphate dehydrogenase,
heterocyclic oxidases such as uricase and xanthine oxidase,
coupled with an enzyme that employs hydrogen peroxide to

15   oxidize a dye precursor such as HRP, lactoperoxidase, or
microperoxidase, biotin/avidin, spin labels, bacteriophage
labels, stable free radicals, and the like.

     Conventional methods are available to bind these
labels covalently to proteins or polypeptides. For

20   instance, coupling agents such as dialdehydes,
carbodiimides, dimaleimides, bis-imidates, bis-diazotized
benzidine, and the like may be used to tag the antibodies
with the above-described fluorescent, chemiluminescent, and
enzyme labels. See, for example, U.S. Pat. Nos. 3,940,475

25   (fluorimetry) and 3,645,090 (enzymes); Hunter et al.,
Nature 194:495-496, 1962; David et al., Biochemistry
13:1014-1021, 1974; Pain et al., J. Immunol. Methods
40:219-230, 1981; and Nygren, J. Histochem. and Cytochem.
30:407-412, 1982. Preferred labels are enzymes such as

30   horseradish peroxidase and alkaline phosphatase.

     The conjugation of such label, including the enzymes,
to the antibody is a standard manipulative procedure for
one of ordinary skill in immunoassay techniques. See, for
example, O'Sullivan et al., "Methods for the Preparation of

35   Enzyme-antibody Conjugates for Use in Enzyme Immunoassay,"
in Methods in Enzymology, ed. J.J. Langone and H. Van
Vunakis, Vol. 73 (Academic Press, New York, New York,
1981), pp. 147-166. Such bonding methods are suitable for
use with C-C CKR-1 or its antibodies, all of which are

proteinaceous.

Immobilization of reagents is required for certain
assay methods. Immobilization entails separating the
binding partner from any analyte that remains free in
5   solution. This conventionally is accomplished by either
insolubilizing the binding partner or analyte analog before
the assay procedure, as by adsorption to a water-insoluble
matrix or surface (Bennich et al., U.S. 3,720,760), by
covalent coupling (for example, using glutaraldehyde cross-
10   linking), or by insolubilizing the partner or analog
afterward, e.g., by immunoprecipitation.

Other assay methods, known as competitive or sandwich
assays, are well established and widely used in the
commercial diagnostics industry.

15   Competitive assays rely on the ability of a tracer
analog to compete with the test sample analyte for a
limited number of binding sites on a common binding
partner. The binding partner generally is insolubilized
before or after the competition and then the tracer and
20   analyte bound to the binding partner are separated from the
unbound tracer and analyte. This separation is
accomplished by decanting (where the binding partner was
preinsolubilized) or by centrifuging (where the binding
partner was precipitated after the competitive reaction).
25   The amount of test sample analyte is inversely proportional
to the amount of bound tracer as measured by the amount of
marker substance. Dose-response curves with known amounts
of analyte are prepared and compared with the test results
to quantitatively determine the amount of analyte present
30   in the test sample. These assays are called ELISA systems
when enzymes are used as the detectable markers.

Another species of competitive assay, called a
"homogeneous" assay, does not require a phase separation.
Here, a conjugate of an enzyme with the analyte is prepared
35   and used such that when anti-analyte binds to the analyte
the presence of the anti-analyte modifies the enzyme
activity. In this case, C-C CKR-1 or its immunologically
active fragments are conjugated with a bifunctional organic
bridge to an enzyme such as peroxidase. Conjugates are

44

selected for use with anti-C-C CKR-1 so that binding of the
anti-C-C CKR-1 inhibits or potentiates the enzyme activity
of the label. This method per se is widely practiced under
the name of EMIT.

5   Steric conjugates are used in steric hindrance methods
for homogeneous assay. These conjugates are synthesized by
covalently linking a low-molecular-weight hapten to a small
analyte so that antibody to hapten substantially is unable
to bind the conjugate at the same time as anti-analyte.

10 Under this assay procedure the analyte present in the test
sample will bind anti-analyte, thereby allowing anti-hapten
to bind the conjugate, resulting in a change in the
character of the conjugate hapten, e.g., a change in
fluorescence when the hapten is a fluorophore.

15   Sandwich assays particularly are useful for the
determination of C-C CKR-1 or C-C CKR-1 antibodies. In
sequential sandwich assays an immobilized binding partner
is used to adsorb test sample analyte, the test sample is
removed as by washing, the bound analyte is used to adsorb

20 labeled binding partner, and bound material is then
separated from residual tracer. The amount of bound tracer
is directly proportional to test sample analyte. In
"simultaneous" sandwich assays the test sample is not
separated before adding the labeled binding partner. A

25 sequential sandwich assay using an anti-C-C CKR-1
monoclonal antibody as one antibody and a polyclonal anti-
C-C CKR-1 antibody as the other is useful in testing
samples for C-C CKR-1 activity.

        The foregoing are merely exemplary diagnostic assays

30 for C-C CKR-1 and antibodies. Other methods now or
hereafter developed for the determination of these analytes
are included within the scope hereof, including the
bioassays described above.

        The following examples are offered by way of

35 illustration and not by way of limitation.

     C. EXPERIMENTAL EXAMPLES

                         Example I

45

Isolation of Orphan Receptors

An "orphan receptor" cloning strategy was employed in
an attempt to isolate cDNAs encoding C-C chemokine
receptors.   The cell surface receptors for the

5   chemoattractants C5a (Gerard, N. P. et al. Nature 349:614-
7, 1991), the bacterial tripeptide fMLP (Boulay, F. et al.
Biochemistry 29:11123-11133, 1990), as well as two
receptors for the C-X-C chemokine IL-8 receptors A and B ·
(IL8rA and IL8rB) have been recently cloned (Holmes, W. E.

10 et al. Science 253:1278-80, 1991; Murphy, P. M. et al.
Science 253:1280-3, 1991) and found to belong to the
superfamily of receptor proteins whose structures are
predicted to transverse the cell membrane seven times
(Dohlman, H. G. et al. Annu. Rev. Biochem. 60:653-88,

15 1991).   Since seven-transmembrane-spanning molecules are
typically linked to G-proteins whose function can be
inhibited by pertussis toxin, we assumed that the receptors
for the C-C chemokine class of proteins would also share
the seven transmembrane architecture.   Accordingly, two

20 degenerate oligonucleotides corresponding to conserved
amino acid sequences in two transmembrane regions (TM) of
the IL8rA, the C5a and the fMLP receptors were synthesized.
The first oligonucleotide corresponded to a region in TM2:
LNLA(L/V)AD(L/F)(L/G) (SEQ ID NO:9) and the second in TM7:

25 NP(I/M)(I/L)Y(A/V)(F/V)·(I/M/A)GQ (SEQ ID NO:10).

These oligomers were then used as primers in RT-PCR
experiments using cDNA substrates from different
hematopoietic cell types known to respond to C-C chemokines
including peripheral blood mononuclear cells (PBMC), and

30 the cell lines U937, HL60 and THP-1. PCR was performed as
follows.   1-2 µg of total RNA from different hematopoietic
cell lines were used as substrates in RT-PCR (Larrick, J.
W. Trends Biotech. 10:146-152, 1992), as recommended by
the supplier (Perkin Elmer, Norwalk, CT). Degenerate

35 oligonucleotides corresponding to conserved regions of
chemoattractant receptors were used in the PCR.   PCR
conditions were as follows: 94ºC for 0.5', 50-55ºC for 0.5'
72ºC for 0.5-1', 30 cycles. PCR products were blunt-end
cloned into the SmaI site of pBS (Stratagene. LaJolla, CA)

46

as previously described (Nguyen, T. et al. Gene 109:211-8,
1991). Plasmid DNA was isolated using the Quiagen kit
(Quiagen Inc., Chatsworth, CA) as recommended by the
supplier. Sequencing was performed with the Sequenase kit
5   (USBC, Cleveland, OH) as recommended by the supplier.

Subcloning and sequencing of the PCR products revealed
the presence of IL8rA, IL8rB, C5a receptor and two novel
clones having characteristics of seven-transmembrane-
segment receptors and marked similarity to the two IL-8
10   receptors. Partial functional characterization of these
orphan receptors revealed that they do not bind to HuMIP-
1α. However, it was noted that these two clones, which
were more related to the IL-8 receptors than to other
seven-transmembrane-spanning receptors, possessed a new
15   conserved amino acid motif at the end of TM3, DRYLAIVHA
(SEQ ID NO:11), which seemed to define a subfamily of IL-8
receptor-related seven-transmembrane-spanning molecules
that excluded the C5a and the fMLP receptors. Therefore, a
second round of RT-PCR/orphan cloning was carried out using
20   the TM2 degenerate oligonucleotide and a DRYLAIVHA (SEQ ID
NO:11) degenerate oligonucleotide. In addition, to
increase the chances of obtaining C-C chemokine receptors,
cDNA was obtained from cultured human monocytes, which bind
radiolabeled HuMIP-1α and from B Cells, which respond
25   chemotactically to HuMIP-1α and used in the PCR reaction.
Cloning and sequencing of these PCR products revealed
several additional unique seven-transmembrane-spanning
receptors.

Monocytes were cultured by standard techniques
30   commonly known in the art. In summary, buffy coat cells
were separated on a Ficoll gradient. Mononuclear cells
recovered from the interface were repeatedly centrifuged to
remove platelets. The monocytes were separated from other
mononuclear cells by adhering them to tissue culture
35   dishes. The non-adherent cells were washed off and the
adherent monocytes were then were cultured for 48-72 hours
before use.

Alternatively, genomic or cDNA could be screened for

the presence of orphan receptor genes by traditional
Southern blot hybridization to the oligomers described
above, or to probes designed from cloned DNA of a seven-
transmembrane spanning protein.

5

Example II
Characterization of C-C CKR-1 DNA

A cDNA corresponding to one clone, JOSH1, was isolated
by screening a λgt10 cDNA library made from PMA (phorbol

10   12-myristate 13-acetate) treated HL60 cells with ^{32}p-
labeled restriction fragments. Phage DNA was isolated and
the inserts of the λgt10 clones were obtained by PCR,
employing 20 cycles with primers flanking the insert and
the enzyme Pfu DNA polymerase (Stratagene, LaJolla, CA), as

15   recommend by the supplier. The PCR products were subcloned
into the SmaI site of pRK5 as described above.

The nucleotide sequence of JOSH1 revealed an open
reading frame of 1065 bases, encoding a protein of 355
amino acids (Figures 1 and 9). The deduced amino acid

20   sequence, provisionally designated as the C-C chemokine
receptor 1 (C-C CKR-1), has key features related to G-
protein-linked receptors of the seven-transmembrane-
spanning receptor superfamily. For example, it has seven
hydrophobic regions predicted to span the cell membrane,

25   and cysteine residues in the first and the second
extracellular loops that are implicated in forming a
disulfide bond (Figure 1). However, certain features of
the predicted C-C CKR-1 protein make it distinct from
classical seven-transmembrane-spanning receptors. The

30   carboxyl-terminus is relatively short and lacks cysteine
residues involved in membrane anchorage via a palmitoylated
moiety (O'Dowd, B. F. et al. J. Biol. Chem. 264:7564-9,
1989) and the segments between the transmembrane domains
are relatively short, a feature consistent in other

35   chemoattractant receptors (Boulay, F. et al. Biochemistry
30:2993-9, 1991; Boulay, F. et al. Biochemistry 29:11123-
11133, 1990; Gerard, N. P. et al. Nature 349:614-7, 1991;
Holmes, W. E. et al. Science 253:1278-80, 1991; Murphy, P.

48

M. et al. Science 253:1280-3, 1991). There are three potential glycosylation sites in the C-C CKR-1 (Figure 1), one in the N-terminus, one in the first cytoplasmic loop and the third in TM6. This latter site is unlikely to be

5   glycosylated since it is predicted to be embedded in the cell membrane. Finally, there is a consensus sequence for a protein kinase C phosphorylation site, at position 192, but this position is predicted to be extracellular.

The deduced amino acid sequence of C-C CKR-1 was

10   compared to other G-protein linked chemoattractant receptors (Figure 1). The IL8rA and IL8rB showed about 32% sequence identity to C-C CKR-1, while the C5a and fMLP receptors showed about 23% identity (Figure 1). A putative seven transmembrane spanning molecule, HUMSTSR, which has

15   been recently been deposited in Genbank (accession #M99293) by Federsspiel, et al., was found to have about 31% identity with C-C CKR-1. The sequence of this molecule is identical to one of the putative receptors isolated in our first attempt at RT-PCR cloning described above. The

20   ligand for HUMSTSR has not yet been identified. The second group of receptors which are as closely related to the C-C CKR-1 are the neuropeptide Y and the angiotensin II receptors (data not shown). Lastly, an open reading frame in the cytomegalovirus genome, designated US28, has about

25   33% identity with the C-C CKR-1 and about 60% identity in the N-terminal region before TM1 with C-C CKR-1.

Example III

Northern and Southern analysis of C-C CKR-1

The expression of the C-C CKR-1 was assessed in a

30   limited panel of hematopoietic cell lines using Northern blot analysis. Northern blot hybridization was performed as follows. Total RNA was isolated using the guanidinium isothiocyanate-CsCl procedure (Sambrook et al., 1989) or by the RNAzol method as recommended by the supplier. Poly A^+

35   RNA was isolated using Dynabeads oligodT (DYNAL, Great Neck, NY) as recommended by the supplier. HL60 mRNA designated "HL60-C" in this report was obtained from Clontech (Palo Alto, CA).   20 μg of total RNA or 5 μg of

49

poly A+ RNA was fractionated on formaldehyde-agarose gels,
blotted to a nitrocellulose membrane and hybridized with
the C-C CKR-1 cDNA (Korneluk, R. G. et al. J. Biol. Chem.
261:8407-8413, 1986).

5   A single band of about 3 kb was detected in pre-
monocytic cell lines, e.g. undifferentiated or
differentiated U937 and HL60 cells (Figure 2), but not in a
commercially available preparation of HL60 RNA (HL60-C,
Figure 2). Lower levels of mRNA were also detected in B
10   cell lines 1788 and Daudi, but little or no RNA was
detected in K562 cells (Figure 2). The highest levels were
detected in PMA-treated THP-1 cells, where a strong signal
was obtained when 20 mg of total RNA was analyzed (Figure
2).

15   Southern blot hybridization was performed as follows.
Human genomic DNA, obtained from Clontech (Palo Alto, CA),
was restriction digested, blotted onto Genescreen® (Dupont)
and hybridized (Neote, K., et al. J. Clin. Invest. 86:1524-
31, 1990) with the C-C CKR-1 cDNA. The hybridization
20   pattern indicated that the C-C CKR-1 gene could be
intronless. Furthermore, it suggested the existence of a
second related gene or possibly a pseudogene. Figures 3A
and 3B represent low and high stringency washes of the same
blot of human genomic DNA which has been digested with
25   several restriction enzymes. Under high stringency
conditions (Figure 3B), single restriction fragments
hybridizing to the C-C CKR-1 cDNA were detected when
genomic DNA was digested with BamHI, HindIII or SacI and,
as predicted from the cDNA sequence, two fragments are
30   detected in DNA digested with EcoRI and PstI. However, low
stringency hybridization revealed the presence of
additional bands that hybridized to the C-C CKR-1 cDNA e.g.
an approximately 7 kb PstI fragment and an approximately
1.6 kb HindIII fragment were detected (Figure 3A). These
35   bands disappeared when the blots were washed under high
stringency conditions while the other bands remained
unchanged between the two washes.

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Example IV

Signaling through the C-C CKR-1 in response to

HuMIP-1α and RANTES

    MCP-1, RANTES, HuMIP-1α and HuMIP-1β induce a rapid

5 and transient increase in intracellular Ca^{++} in human
monocytes (Rollins, B. J. et al. Blood 78:1112-6, 1991;
Sozzani, S. et al. J. Immunol. 147:2215-21, 1991). To
determine if C-C CKR-1 was a functional C-C chemokine
receptor, it was transiently expressed in human kidney 293

10 cells and intracellular Ca^{++} levels in response to
different C-C chemokines were measured.

    Recombinant RANTES was expressed in E. coli and
purified as described by Kuna et al.(J. Immunol. 149:636-
42, 1992b). HuMIP-1α and HuMIP-1β was expressed in E.

15 coli, and purified as described by Rot et al. (J. Exp.
Med., in press). HuMIP-1α was iodinated by the
chloroglycoluril method (Fraker et al. Biochem. Biophys.
Res. Comm. 80:849-857, 1978) to an initial specific
activity of 472 Ci/mmol. The labeled HuMIP-1a was purified

20 by a combination of gel filtration and reversed-phase HPLC.
The ^{125}I-labeled RANTES, specific activity 2200 Ci/mmol,
was obtained from Dupont/NEN (Boston, MASS). Recombinant
MCP-1 and murine MIP-1α were obtained from Peprotech (Rocky
Hill, NJ).

25   Human embryonic kidney 293 cells were transfected with
10-20 μg of plasmid DNA by the calcium-phosphate method as
described (Schall, T. J. et al. Eur. J. Immunol. 22:1477-
81, 1992) or the modified calcium phosphate method (Chen,
C. et al. Mol. Cell. Biol. 7:2745-2752, 1987). The

30 transfected cells were assayed after transient expression
for 12-24 hours.

    Intracellular Ca^{++} measurements were done on the SLM
8000C essentially as described (Naccache, P. H. et al. J.
Immunol. 142:2438-44, 1989), with minor modifications:

35 INDO-1-AM, was used at 20 mg/ml final concentration and 2-4
$X10^6$ cells were used per assay.

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A 100 nM dose of RANTES, HuMIP-1α, HuMIP-1β and MCP-1
was initially used as a first approximation of the maximum
physiologically-relevant concentration. When transfected
cells, loaded with the calcium probe INDO-1-AM, were
5   challenged with 100 nM of either HuMIP-1α or RANTES, a
rapid increase in intracellular Ca^{++} was observed (Figure
4). Challenge with the same dose of MCP-1 or HuMIP-1β
produced little detectable Ca^{++} flux (data not shown).
Control cells transfected with vector alone or vector
10   containing C-C CKR-1 in the opposite (non-coding)
orientation did not respond to any of the ligands (data not
shown). The signaling responses were dose dependent, 10 nM
of HuMIP-1α and 100 nM of RANTES were sufficient to give a
maximal or near maximal response (Figure 4A and 4B).

15   Rapid, successive exposure to the same ligand is known
to desensitize the signaling capacity of G-protein linked
receptors (Schild, H. O. (1973), "Receptor classification
with special reference to β-adrenergic receptors," In Drug
Receptors, H.P. Rang, ed. (University Press), pp. 29-36).
20   In addition, desensitization can also occur when the two
different agonists signal through the same receptor.
HuMIP-1α clearly blocks the ability of the C-C CKR-1
receptor to transmit a second Ca^{++} signal when HuMIP-1α is
added to transfected cells twice in succession (Figure 5A).
25   Similarly, RANTES blocks the response to a second challenge
at the same concentration (Figure 5B). When HuMIP-1α is
added first, it blocks the response to RANTES, indicating
that complete desensitization has occurred (Figure 5C).
However, challenging C-C CKR-1 cDNA transfected cells with
30   250 nM RANTES did not prevent a subsequent Ca^{++} flux by the
addition of 100 nM HuMIP-1α (Figure 5D), indicating that
receptor desensitization had not occurred. However, the
subsequent Ca^{++} flux by HuMIP-1α is reduced, from a
intracellular Ca^{++} change of about 70 nM (Figure 5A and C)
35   to about 40 nM (Figure 5D), suggesting that a partial
desensitization has occurred.

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Example V

Binding of HuMIP-1α and RANTES to C-C CKR-1

In order to further investigate the interaction of
HuMIP-1α and RANTES to the cloned C-C CKR-1, direct binding
5   experiments with ^{125}I-labeled ligands were carried out.

Binding assays were performed as described previously
(Horuk, R. et al. J. Biol. Chem. 262:16275-16278, 1987).
Transfected cells (2 x 10^6 cells per ml) were incubated
with radiolabeled ligands and varying concentrations of
10   unlabeled ligands at 4 °C for 2 hours. The incubation was
terminated by removing aliquots from the cell suspension
and separating cells from buffer by centrifugation through
a silicon/paraffin oil mixture as described previously
(Robb, R. J. et al. J. Exp. Med. 160:1126-1146, 1984).
15   Non-specific binding was determined in the presence of 1 μM
unlabeled ligand. Individual assay determinations,
representative of at least three separate experiments were
plotted. The binding data were curve fit with the computer
program LIGAND (Munson, P. J. et al. Anal. Biochem.
20   107:220-239, 1980) modified for the IBM PC (McPherson, G.
A. Comp. Prog. Biomed. 17:107-114, 1983) to determine the
affinity (K_d), number of sites, and nonspecific binding.
The curves shown are the binding isotherms determined by
LIGAND.

25   Thus, when 293 cells transiently expressing C-C CKR-1
were incubated with ^{125}I-HuMIP-1α and increasing
concentrations of unlabeled HuMIP-1α, displaceable binding
of ^{125}I-HuMIP-1α to the C-C CKR-1 was observed (Figure 6A).
Scatchard analysis showed a dissociation constant (K_d) of
30   5:1±0.3nM and about 130000 sites/cell. This K_d is within
the range of that determined for HuMIP-1α binding to human
monocytes and suggests that C-C CKR-1 is at least one of
the HuMIP-1α receptors present on monocytes. Direct
binding of RANTES to the C-C CKR-1 however could not be
35   accomplished, i.e., ^{125}I-RANTES could not be displaced by
unlabeled RANTES. Interestingly, as the amount of
unlabeled RANTES was increased in the binding assay, a

53

concomitant increase of ^{125}I-RANTES bound to cells was
observed (data not shown). Since the C-C CKR-1 transduces
a signal (mobilizes Ca^{++}) in response to RANTES (Figure 4),
and therefore, the ligand must be binding to the cloned

5 receptor, the reason for the unusual binding profile
observed for ^{125}I-RANTES is not clear. Similar binding
phenomenon are obtained if other target cells responding to
RANTES are used. Interestingly, ^{125}I-RANTES could be
displaced by unlabeled HuMIP-1α on 293 cells transiently

10 expressing C-C CKR-1 (Figure 6B). Scatchard analysis of
this heterologous displacement suggested a K_d of 7.6±1.5nM
(about 350000 sites/cell) and is consistent with the K_d of
HuMIP-1α binding data described above. These observations
suggested to us initially that the C-C CKR-1 binds to

15 HuMIP-1α and RANTES and subsequently transduces a signal by
increasing the intracellular Ca^{++} levels

<u>Example VI</u>

<u>Displacement of HuMIP-1α by Heterologous Chemokines</u>

To further characterize the binding properties of the
20 C-C CKR-1, and in particular to attempt to define a K_d for
RANTES binding to the cloned C-C CKR-1, heterologous
displacement of ^{125}I-HuMIP-1α was done with RANTES and also
HuMIP-1β, MCP-1, IL-8, and murine MIP-1α. Interestingly,
all C-C chemokines (RANTES, MIP-1b and MCP-1) displaced
25 ^{125}I-HuMIP-1α, but the C-X-C chemokine IL-8 did not (Figure
7). The K_d for RANTES, HuMIP-1b and MCP-1, and murine MIP-
1α as determined from Scatchard analysis is 468±280,
232±70.1, 122±39.3, and 4.2±2.7 nM respectively, and in
each case about 250000-350000 sites/cell are present
30 (Figure 7). These results revealed a broad ligand
specificity of the C-C CKR-1, including some lack of
species specificity in binding. More importantly, it
suggested that the binding affinity between the ligand and
the receptor does not predict the signaling efficacy
35 resulting from that interaction, i.e. although HuMIP-1β and
MCP-1 bind with a higher affinity then does RANTES, 100 nM
of MIP-1β or MCP-1 do not transmit a signal via the cloned
receptor, whereas RANTES does (Figure 4).

54

Example VII

Calcium Mobilization in Response to HuMIP-1β and MCP-1

To determine the relevance of HuMIP-1β and MCP-1

binding to C-C CKR-1, C-C CKR-1 cDNA transfected 293 cells
were challenged with high doses of HuMIP-1β and MCP-1 and

5   intracellular Ca^{++} levels were determined. A concentration
of 1 mM of the recombinant MCP-1 had to be used to produce
a Ca^{++} flux. This Ca^{++} flux, however, was only about 20%
of the maximum response obtained by HuMIP-1α or RANTES
(Figure 8). Lower concentrations gave an almost

10   undetectable flux (data not shown). Control experiments
utilizing THP-1 cells and 50-100 nM of MCP-1 gave the
expected strong Ca^{++} fluxes (a transient increase of 200-
300 nM Ca^{++}, data not shown). Similarly, 250 nM of HuMIP-
1β was needed to produce a Ca^{++} flux in 293 cells

15   transiently expressing C-C CKR-1 that was about 20% of the
maximal signal obtained by HuMIP-1α or RANTES (Figure 8).
These results support the suggestion that the binding
affinities HuMIP-1β and MCP-1 do not reflect their
signaling capabilities.

20   ## Example VIII

Binding of HuMIP-1α to 293 Cells Expressing the Protein
Encoded by US28

Since the amino acid sequence of C-C CKR-1 is about
35% identical to an open reading frame in the human CMV

25   genome designated as US28, we sought to determine if the
protein encoded by this open reading frame would bind HuMIP-
1α. The US28 open reading frame was amplified from a stock
of Towne strain cytomegalovirus (a kind gift of Dr. Philip
Dormitzer) with primers flanking the coding region using PCR

30   and the thermostable DNA polymerase Pfu. The PCR product was
subcloned into the expression vector pRK5 and its identity
confirmed by sequencing. Plasmid DNA containing US28 in the
sense orientation and the opposite, antisense orientation was
transfected into 293 cells. Transfected cells were then used

35   to determine binding of radiolabeled HuMIP-1α. 293 cells
transfected with the US28 coding sequence in the sense

orientation bound ^{125}I-HuMIP-1α, whereas negligible binding was obtained in cells transfected with the antisense orientation (Figure 10). The radiolabeled HuMIP-1α could also be displaced with 1 μM of murine MIP-1α, HuMIP-1β, RANTES, and

5   MCP-1, but not by the C-X-C chemokine, IL-8 (Figure 10). These results indicate that the protein encoded by US28 specifically binds C-C chemokines and not C-X-C chemokines.

56

SEQUENCE LISTING

(1) GENERAL INFORMATION:

  (i) APPLICANT: GENENTECH, INC.

  (ii) TITLE OF INVENTION: CC-CHEMOKINE RECEPTOR

 (iii) NUMBER OF SEQUENCES: 11

  (iv) CORRESPONDENCE ADDRESS:
     (A) ADDRESSEE: Genentech, Inc.
     (B) STREET: 460 Point San Bruno Blvd
     (C) CITY: South San Francisco
     (D) STATE: California
     (E) COUNTRY: USA
     (F) ZIP: 94080

  (v) COMPUTER READABLE FORM:
     (A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
     (B) COMPUTER: IBM PC compatible
     (C) OPERATING SYSTEM: PC-DOS/MS-DOS
     (D) SOFTWARE: patin (Genentech)

  (vi) CURRENT APPLICATION DATA:
     (A) APPLICATION NUMBER:
     (B) FILING DATE:
     (C) CLASSIFICATION:

 (vii) PRIOR APPLICATION DATA:
     (A) APPLICATION NUMBER:
     (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
     (A) NAME: Fitts, Renee A.
     (B) REGISTRATION NUMBER: 35,136
     (C) REFERENCE/DOCKET NUMBER: 806

  (ix) TELECOMMUNICATION INFORMATION:
     (A) TELEPHONE: 415/225-1489
     (B) TELEFAX: 415/952-9881
     (C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

  (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 355 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Glu Thr Pro Asn Thr Thr Glu Asp Tyr Asp Thr Thr Thr Glu
1   5   10   15

57

```
Phe Asp Tyr Gly Asp Ala Thr Pro Cys Gln Lys Val Asn Glu Arg
              20               25               30

Ala Phe Gly Ala Gln Leu Leu Pro Pro Leu Tyr Ser Leu Val Phe
              35               40               45

Val Ile Gly Leu Val Gly Asn Ile Leu Val Val Leu Val Leu Val
              50               55               60

Gln Tyr Lys Arg Leu Lys Asn Met Thr Ser Ile Tyr Leu Leu Asn
              65               70               75

Leu Ala Ile Ser Asp Leu Leu Phe Leu Phe Thr Leu Pro Phe Trp
              80               85               90

Ile Asp Tyr Lys Leu Lys Asp Asp Trp Val Phe Gly Asp Ala Met
              95              100              105

Cys Lys Ile Leu Ser Gly Phe Tyr Tyr Thr Gly Leu Tyr Ser Glu
             110              115              120

Ile Phe Phe Ile Ile Leu Leu Thr Ile Asp Arg Tyr Leu Ala Ile
             125              130              135

Val His Ala Val Phe Ala Leu Arg Ala Arg Thr Val Thr Phe Gly
             140              145              150

Val Ile Thr Ser Ile Ile Ile Trp Ala Leu Ala Ile Leu Ala Ser
             155              160              165

Met Pro Gly Leu Tyr Phe Ser Lys Thr Gln Trp Glu Phe Thr His
             170              175              180

His Thr Cys Ser Leu His Phe Pro His Glu Ser Leu Arg Glu Trp
             185              190              195

Lys Leu Phe Gln Ala Leu Lys Leu Asn Leu Phe Gly Leu Val Leu
             200              205              210

Pro Leu Leu Val Met Ile Ile Cys Tyr Thr Gly Ile Ile Lys Ile
             215              220              225

Leu Leu Arg Arg Pro Asn Glu Lys Lys Ser Lys Ala Val Arg Leu
             230              235              240

Ile Phe Val Ile Met Ile Ile Phe Phe Leu Phe Trp Thr Pro Tyr
             245              250              255

Asn Leu Thr Ile Leu Ile Ser Val Phe Gln Asp Phe Leu Phe Thr
             260              265              270

His Glu Cys Glu Gln Ser Arg His Leu Asp Leu Ala Val Gln Val
             275              280              285

Thr Glu Val Ile Ala Tyr Thr His Cys Cys Val Asn Pro Val Ile
             290              295              300
```

58

```
Tyr Ala Phe Val Gly Glu Arg Phe Arg Lys Tyr Leu Arg Gln Leu
            305                 310                 315

Phe His Arg Arg Val Ala Val His Leu Val Lys Trp Leu Pro Phe
            320                 325                 330

Leu Ser Val Asp Arg Leu Glu Arg Val Ser Ser Thr Ser Pro Ser
            335                 340                 345

Thr Gly Glu His Glu Leu Ser Ala Gly Phe
            350                 355
```

(2) INFORMATION FOR SEQ ID NO:2:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 352 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Gly Ile Ser Ile Tyr Thr Ser Asp Asn Tyr Thr Glu Glu
  1               5               10                  15

Met Gly Ser Gly Asp Tyr Asp Ser Met Lys Glu Pro Cys Phe Arg
            20                  25                  30

Glu Glu Asn Ala Asn Phe Asn Lys Ile Phe Leu Pro Thr Ile Tyr
            35                  40                  45

Ser Ile Ile Phe Leu Thr Gly Ile Val Gly Asn Gly Leu Val Ile
            50                  55                  60

Leu Val Met Gly Tyr Gln Lys Lys Leu Arg Ser Met Thr Asp Lys
            65                  70                  75

Tyr Arg Leu His Leu Ser Val Ala Asp Leu Leu Phe Val Ile Thr
            80                  85                  90

Leu Pro Phe Trp Ala Val Asp Ala Val Ala Asn Trp Tyr Phe Gly
            95                  100                 105

Asn Phe Leu Cys Lys Ala Val His Val Ile Tyr Thr Val Asn Leu
            110                 115                 120

Tyr Ser Ser Val Leu Ile Leu Ala Phe Ile Ser Leu Asp Arg Tyr
            125                 130                 135

Leu Ala Ile Val His Ala Thr Asn Ser Gln Arg Pro Arg Lys Leu
            140                 145                 150

Leu Ala Glu Lys Val Val Tyr Val Gly Val Trp Ile Pro Ala Leu
            155                 160                 165

Leu Leu Thr Ile Pro Asp Phe Ile Phe Ala Asn Val Ser Glu Ala
            170                 175                 180
```

```
Asp Asp Arg Tyr Ile Cys Asp Arg Phe Tyr Pro Asn Asp Leu Trp
            185             190             195

Val Val Val Phe Gln Phe Gln His Ile Met Val Gly Leu Ile Leu
            200             205             210

Pro Gly Ile Val Ile Leu Ser Cys Tyr Cys Ile Ile Ile Ser Lys
            215             220             225

Leu Ser His Ser Lys Gly His Gln Lys Arg Lys Ala Leu Lys Thr
            230             235             240

Thr Val Ile Leu Ile Leu Ala Phe Phe Ala Cys Trp Leu Pro Tyr
            245             250             255

Tyr Ile Gly Ile Ser Ile Asp Ser Phe Ile Leu Leu Glu Ile Ile
            260             265             270

Lys Gln Gly Cys Glu Phe Glu Asn Thr Val His Lys Trp Ile Ser
            275             280             285

Ile Thr Glu Ala Leu Ala Phe Phe His Cys Cys Leu Asn Pro Ile
            290             295             300

Leu Tyr Ala Phe Leu Gly Ala Lys Phe Lys Thr Ser Ala Gln His
            305             310             315

Ala Leu Thr Ser Val Ser Arg Gly Ser Ser Leu Lys Ile Leu Ser
            320             325             330

Lys Gly Lys Arg Gly Gly His Ser Ser Val Ser Thr Glu Ser Glu
            335             340             345

Ser Ser Ser Phe His Ser Ser
            350     352
```

(2) INFORMATION FOR SEQ ID NO:3:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 350 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ser Asn Ile Thr Asp Pro Gln Met Trp Asp Phe Asp Asp Leu
1               5               10              15

Asn Phe Thr Gly Met Pro Pro Ala Asp Glu Asp Tyr Ser Pro Cys
            20              25              30

Met Leu Glu Thr Glu Thr Leu Asn Lys Tyr Val Val Ile Ile Ala
            35              40              45

Tyr Ala Leu Val Phe Leu Leu Ser Leu Leu Gly Asn Ser Leu Val
            50              55              60
```

60

Met Leu Val Ile Leu Tyr Ser Arg Val Gly Arg Ser Val Thr Asp
                65   70   75

Val Tyr Leu Leu Asn Leu Ala Leu Ala Asp Leu Leu Phe Ala Leu
                80   85   90

Thr Leu Pro Ile Trp Ala Ala Ser Lys Val Asn Gly Trp Ile Phe
                95   100   105

Gly Thr Phe Leu Cys Lys Val Val Ser Leu Leu Lys Glu Val Asn
                110   115   120

Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp Arg
                125   130   135

Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu Thr Gln Lys Arg
                140   145   150

His Leu Val Lys Phe Val Cys Leu Gly Cys Trp Gly Leu Ser Met
                155   160   165

Asn Leu Ser Leu Pro Phe Phe Leu Phe Arg Gln Ala Tyr His Pro
                170   175   180

Asn Asn Ser Ser Pro Val Cys Tyr Glu Val Leu Gly Asn Asp Thr
                185   190   195

Ala Lys Trp Arg Met Val Leu Arg Ile Leu Pro His Thr Phe Gly
                200   205   210

Phe Ile Val Pro Leu Phe Val Met Leu Phe Cys Tyr Gly Phe Thr
                215   220   225

Leu Arg Thr Leu Phe Lys Ala His Met Gly Gln Lys His Arg Ala
                230   235   240

Met Arg Val Ile Phe Ala Val Val Leu Ile Phe Leu Leu Cys Trp
                245   250   255

Leu Pro Tyr Asn Leu Val Leu Leu Ala Asp Thr Leu Met Arg Thr
                260   265   270

Gln Val Ile Gln Glu Thr Cys Glu Arg Arg Asn Asn Ile Gly Arg
                275   280   285

Ala Leu Asp Ala Thr Glu Ile Leu Gly Phe Leu His Ser Cys Leu
                290   295   300

Asn Pro Ile Ile Tyr Ala Phe Ile Gly Gln Asn Phe Arg His Gly
                305   310   315

Phe Leu Lys Ile Leu Ala Met His Gly Leu Val Ser Lys Glu Phe
                320   325   330

Leu Ala Arg His Arg Val Thr Ser Tyr Thr Ser Ser Ser Val Asn
                335   340   345

Val Ser Ser Asn Leu
                350

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 355 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Glu Ser Asp Ser Phe Glu Asp Phe Trp Lys Gly Glu Asp Leu
  1   5   10   15

Ser Asn Tyr Ser Tyr Ser Ser Thr Leu Pro Pro Phe Leu Leu Asp
                20   25   30

Ala Ala Pro Cys Glu Pro Glu Ser Leu Glu Ile Asn Lys Tyr Phe
                35   40   45

Val Val Ile Ile Tyr Ala Leu Val Phe Leu Leu Ser Leu Leu Gly
                50   55   60

Asn Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg Val Gly Arg
                65   70   75

Ser Val Thr Asp Val Tyr Leu Leu Asn Leu Ala Leu Ala Asp Leu
                80   85   90

Leu Phe Ala Leu Thr Leu Pro Ile Trp Ala Ala Ser Lys Val Asn
                95   100   105

Gly Trp Ile Phe Gly Thr Phe Leu Cys Lys Val Val Ser Leu Leu
                110   115   120

Lys Glu Val Asn Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile
                125   130   135

Ser Val Asp Arg Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu
                140   145   150

Thr Gln Lys Arg Tyr Leu Val Lys Phe Ile Cys Leu Ser Ile Trp
                155   160   165

Gly Leu Ser Leu Leu Leu Ala Leu Pro Val Leu Leu Phe Arg Arg
                170   175   180

Thr Val Tyr Ser Ser Asn Val Ser Pro Ala Cys Tyr Glu Asp Met
                185   190   195

Gly Asn Asn Thr Ala Asn Trp Arg Met Leu Leu Arg Ile Leu Pro
                200   205   210

Gln Ser Phe Gly Phe Ile Val Pro Leu Leu Ile Met Leu Phe Cys
                215   220   225

62

```
Tyr Gly Phe Thr Leu Arg Thr Leu Phe Lys Ala His Met Gly Gln
              230             235                         240

Lys His Arg Ala Met Arg Val Ile Phe Ala Val Val Leu Ile Phe
              245             250                         255

Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu Leu Ala Asp Thr
              260             265                         270

Leu Met Arg Thr Gln Val Ile Gln Glu Thr Cys Glu Arg Arg Asn
              275             280                         285

His Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly Ile Leu
              290             295                         300

His Ser Cys Leu Asn Pro Leu Ile Tyr Ala Phe Ile Gly Gln Lys
              305             310                         315

Phe Arg His Gly Leu Leu Lys Ile Leu Ala Ile His Gly Leu Ile
              320             325                         330

Ser Lys Asp Ser Leu Pro Lys Asp Ser Arg Pro Ser Phe Val Gly
              335             340                         345

Ser Ser Ser Gly His Thr Ser Thr Thr Leu
              350                 355
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 350 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Asn Ser Phe Asn Tyr Thr Thr Pro Asp Tyr Gly His Tyr Asp
1               5                 10                        15

Asp Lys Asp Thr Leu Asp Leu Asn Thr Pro Val Asp Lys Thr Ser
              20              25                          30

Asn Thr Leu Arg Val Pro Asp Ile Leu Ala Leu Val Ile Phe Ala
              35              40                          45

Val Val Phe Leu Val Gly Val Leu Gly Asn Ala Leu Val Val Trp
              50              55                          60

Val Thr Ala Phe Glu Ala Lys Arg Thr Ile Asn Ala Ile Trp Phe
              65              70                          75

Leu Asn Leu Ala Val Ala Asp Phe Leu Ser Cys Leu Ala Leu Pro
              80              85                          90

Ile Leu Phe Thr Ser Ile Val Gln His His His Trp Pro Phe Gly
              95              100                        105
```

63

```
Gly Ala Ala Cys Ser Ile Leu Pro Ser Leu Ile Leu Leu Asn Met
                110           115           120

Tyr Ala Ser Ile Leu Leu Leu Ala Thr Ile Ser Ala Asp Arg Phe
                125           130           135

Leu Leu Val Phe Lys Pro Ile Trp Cys Gln Asn Phe Arg Gly Ala
                140           145           150

Gly Leu Ala Trp Ile Ala Cys Ala Val Ala Trp Gly Leu Ala Leu
                155           160           165

Leu Leu Thr Ile Pro Ser Phe Leu Tyr Arg Val Val Arg Glu Glu
                170           175           180

Tyr Phe Pro Pro Lys Val Leu Cys Gly Val Asp Tyr Ser His Asp
                185           190           195

Lys Arg Arg Glu Arg Ala Val Ala Ile Val Arg Leu Val Leu Gly
                200           205           210

Phe Leu Trp Pro Leu Leu Thr Leu Thr Ile Cys Tyr Thr Phe Ile
                215           220           225

Leu Leu Arg Thr Trp Ser Arg Arg Ala Thr Arg Ser Thr Lys Thr
                230           235           240

Leu Lys Val Val Val Ala Val Val Ala Ser Phe Phe Ile Phe Trp
                245           250           255

Leu Pro Tyr Gln Val Thr Gly Ile Met Met Ser Phe Leu Glu Pro
                260           265           270

Ser Ser Pro Thr Phe Leu Leu Leu Asn Lys Leu Asp Ser Leu Cys
                275           280           285

Val Ser Phe Ala Tyr Ile Asn Cys Cys Ile Asn Pro Ile Ile Tyr
                290           295           300

Val Val Ala Gly Gln Gly Phe Gln Gly Arg Leu Arg Lys Ser Leu
                305           310           315

Pro Ser Leu Leu Arg Asn Val Leu Thr Glu Glu Ser Val Val Arg
                320           325           330

Glu Ser Lys Ser Phe Thr Arg Ser Thr Val Asp Thr Met Ala Gln
                335           340           345

Lys Thr Gln Ala Val
                350
```

64

(2) INFORMATION FOR SEQ ID NO:6:

      (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 350 amino acids
           (B) TYPE: amino acid
           (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Thr Asn Ser Ser Leu Pro Thr Asn Ile Ser Gly Gly Thr
 1   5   10   15

Pro Ala Val Ser Ala Gly Tyr Leu Phe Leu Asp Ile Ile Thr Tyr
                20   25   30

Leu Val Phe Ala Val Thr Phe Val Leu Gly Val Leu Gly Asn Gly
                35   40   45

Leu Val Ile Trp Val Ala Gly Phe Arg Met Thr His Thr Val Thr
                50   55   60

Thr Ile Ser Tyr Leu Asn Leu Ala Val Ala Asp Phe Cys Phe Thr
                65   70   75

Ser Thr Leu Pro Phe Phe Met Val Arg Lys Ala Met Gly Gly His
                80   85   90

Trp Pro Phe Gly Trp Phe Leu Cys Lys Phe Val Phe Thr Ile Val
                95   100   105

Asp Ile Asn Leu Phe Gly Ser Val Phe Leu Ile Ala Leu Ile Ala
                110   115   120

Leu Asp Arg Cys Val Cys Val Leu His Pro Val Trp Thr Gln Asn
                125   130   135

His Arg Thr Val Ser Leu Ala Lys Lys Val Ile Ile Gly Pro Trp
                140   145   150

Val Met Ala Leu Leu Leu Thr Leu Pro Val Ile Ile Arg Val Thr
                155   160   165

Thr Val Pro Gly Lys Thr Gly Thr Val Ala Cys Thr Phe Asn Phe
                170   175   180

Ser Pro Trp Thr Asn Asp Pro Lys Glu Arg Ile Asn Val Ala Val
                185   190   195

Ala Met Leu Thr Val Arg Gly Ile Ile Arg Phe Ile Ile Gly Phe
                200   205   210

Ser Ala Pro Met Ser Ile Val Ala Val Ser Tyr Gly Leu Ile Ala
                215   220   225

Thr Lys Ile His Lys Gln Gly Leu Ile Lys Ser Ser Arg Pro Leu
                230   235   240

65

```
Arg Val Leu Ser Phe Val Ala Ala Ala Phe Phe Leu Cys Trp Ser
              245                     250                 255

Pro Tyr Gln Val Val Ala Leu Ile Ala Thr Val Arg Ile Arg Glu
              260                     265                 270

Leu Leu Gln Gly Met Tyr Lys Glu Ile Gly Ile Ala Val Asp Val
              275                     280                 285

Thr Ser Ala Leu Ala Phe Phe Asn Ser Cys Leu Asn Pro Met Leu
              290                     295                 300

Tyr Val Phe Met Gly Gln Asp Phe Arg Glu Arg Leu Ile His Ala
              305                     310                 315

Leu Pro Ala Ser Leu Glu Arg Ala Leu Thr Glu Asp Ser Thr Gln
              320                     325                 330

Thr Ser Asp Thr Ala Thr Asn Ser Thr Leu Pro Ser Ala Glu Val
              335                     340                 345

Glu Leu Gln Ala Lys
              350
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 323 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Thr Pro Thr Thr Thr Thr Ala Glu Leu Thr Thr Glu Phe Asp
 1             5               10                  15

Tyr Asp Glu Asp Ala Thr Pro Cys Val Phe Thr Asp Val Leu Asn
              20                  25                  30

Gln Ser Lys Pro Val Thr Leu Phe Leu Tyr Gly Val Val Phe Leu
              35                  40                  45

Phe Gly Ser Ile Gly Asn Phe Leu Val Ile Phe Thr Ile Thr Trp
              50                  55                  60

Arg Arg Arg Ile Gln Cys Ser Gly Asp Val Tyr Phe Ile Asn Leu
              65                  70                  75

Ala Ala Ala Asp Leu Leu Phe Val Cys Thr Leu Pro Leu Trp Met
              80                  85                  90

Gln Tyr Leu Leu Asp His Asn Ser Leu Ala Ser Val Pro Cys Thr
              95                  100                 105

Leu Leu Thr Ala Cys Phe Tyr Val Ala Met Phe Ala Ser Leu Cys
              110                 115                 120
```

66

```
Phe Ile Thr Glu Ile Ala Leu Asp Arg Tyr Tyr Ala Ile Val Tyr
              125               130               135

Met Arg Tyr Arg Pro Val Lys Gln Ala Cys Leu Phe Ser Ile Phe
              140               145               150

Trp Trp Ile Phe Ala Val Ile Ile Ala Ile Pro His Phe Met Val
              155               160               165

Val Thr Lys Lys Asp Asn Gln Cys Met Thr Asp Tyr Asp Tyr Leu
              170               175               180

Glu Val Ser Tyr Pro Ile Ile Leu Asn Val Glu Leu Met Leu Gly
              185               190               195

Ala Phe Val Ile Pro Leu Ser Val Ile Ser Tyr Cys Tyr Tyr Arg
              200               205               210

Ile Ser Arg Ile Val Ala Val Ser Gln Ser Arg His Lys Gly Arg
              215               220               225

Ile Val Arg Val Leu Ile Ala Val Val Leu Val Phe Ile Ile Phe
              230               235               240

Trp Leu Pro Tyr His Leu Thr Leu Phe Val Asp Thr Leu Lys Leu
              245               250               255

Leu Lys Trp Ile Ser Ser Ser Cys Glu Phe Glu Arg Ser Leu Lys
              260               265               270

Arg Ala Leu Ile Leu Thr Glu Ser Leu Ala Phe Cys His Cys Cys
              275               280               285

Leu Asn Pro Leu Leu Tyr Val Phe Val Gly Thr Lys Phe Arg Lys
              290               295               300

Asn Tyr Thr Val Cys Trp Pro Ser Phe Ala Ser Asp Ser Phe Pro
              305               310               315

Ala Met Tyr Pro Gly Thr Thr Ala
              320         323
```

(2) INFORMATION FOR SEQ ID NO:8:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 1495 bases
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
ATG GAA ACT CCA AAC ACC ACA GAG GAC TAT GAC ACG 36
Met Glu Thr Pro Asn Thr Thr Glu Asp Tyr Asp Thr
 1               5                   10
```

67

```
ACC ACA GAG TTT GAC TAT GGG GAT GCA ACT CCG TGC CAG 75
Thr Thr Glu Phe Asp Tyr Gly Asp Ala Thr Pro Cys Gln
        15              20                  25

AAG GTG AAC GAG AGG GCC TTT GGG GCC CAA CTG CTG CCC 114
Lys Val Asn Glu Arg Ala Phe Gly Ala Gln Leu Leu Pro
            30                  35

CCT CTG TAC TCC TTG GTA TTT GTC ATT GGC CTG GTT GGA 153
Pro Leu Tyr Ser Leu Val Phe Val Ile Gly Leu Val Gly
    40              45                  50

AAC ATC CTG GTG GTC CTG GTC CTT GTG CAA TAC AAG AGG 192
Asn Ile Leu Val Val Leu Val Leu Val Gln Tyr Lys Arg
            55                  60

CTA AAA AAC ATG ACC AGC ATC TAC CTC CTG AAC CTG GCC 231
Leu Lys Asn Met Thr Ser Ile Tyr Leu Leu Asn Leu Ala
65                  70                  75

ATT TCT GAC CTG CTC TTC CTG TTC ACG CTT CCC TTC TGG 270
Ile Ser Asp Leu Leu Phe Leu Phe Thr Leu Pro Phe Trp
        80                  85                  90

ATC GAC TAC AAG TTG AAG GAT GAC TGG GTT TTT GGT GAT 309
Ile Asp Tyr Lys Leu Lys Asp Asp Trp Val Phe Gly Asp
                95                  100

GCC ATG TGT AAG ATC CTC TCT GGG TTT TAT TAC ACA GGC 348
Ala Met Cys Lys Ile Leu Ser Gly Phe Tyr Tyr Thr Gly
    105                 110                 115

TTG TAC AGC GAG ATC TTT TTC ATC ATC CTG CTG ACG ATT 387
Leu Tyr Ser Glu Ile Phe Phe Ile Ile Leu Leu Thr Ile
            120                 125

GAC AGG TAC CTG GCC ATC GTC CAC GCC GTG TTT GCC TTG 426
Asp Arg Tyr Leu Ala Ile Val His Ala Val Phe Ala Leu
130                 135                 140

CGG GCA CGG ACC GTC ACT TTT GGT GTC ATC ACC AGC ATC 465
Arg Ala Arg Thr Val Thr Phe Gly Val Ile Thr Ser Ile
        145                 150                 155

ATC ATT TGG GCC CTG GCC ATC TTG GCT TCC ATG CCA GGC 504
Ile Ile Trp Ala Leu Ala Ile Leu Ala Ser Met Pro Gly
                160                 165

TTA TAC TTT TCC AAG ACC CAA TGG GAA TTC ACT CAC CAC 543
Leu Tyr Phe Ser Lys Thr Gln Trp Glu Phe Thr His His
    170                 175                 180

ACC TGC AGC CTT CAC TTT CCT CAC GAA AGC CTA CGA GAG 582
Thr Cys Ser Leu His Phe Pro His Glu Ser Leu Arg Glu
            185                 190
```

68

```
TGG AAG CTG TTT CAG GCT CTG AAA CTG AAC CTC TTT GGG 621
Trp Lys Leu Phe Gln Ala Leu Lys Leu Asn Leu Phe Gly
195             200             205

CTG GTA TTG CCT TTG TTG GTC ATG ATC ATC TGC TAC ACA 660
Leu Val Leu Pro Leu Leu Val Met Ile Ile Cys Tyr Thr
        210             215             220

GGG ATT ATA AAG ATT CTG CTA AGA CGA CCA AAT GAG AAG 699
Gly Ile Ile Lys Ile Leu Leu Arg Arg Pro Asn Glu Lys
                225             230

AAA TCC AAA GCT GTC CGT TTG ATT TTT GTC ATC ATG ATC 738
Lys Ser Lys Ala Val Arg Leu Ile Phe Val Ile Met Ile
    235             240             245

ATC TTT TTT CTC TTT TGG ACC CCC TAC AAT TTG ACT ATA 777
Ile Phe Phe Leu Phe Trp Thr Pro Tyr Asn Leu Thr Ile
        250             255

CTT ATT TCT GTT TTC CAA GAC TTC CTG TTC ACC CAT GAG 816
Leu Ile Ser Val Phe Gln Asp Phe Leu Phe Thr His Glu
260             265             270

TGT GAG CAG AGC AGA CAT TTG GAC CTG GCT GTG CAA GTG 855
Cys Glu Gln Ser Arg His Leu Asp Leu Ala Val Gln Val
        275             280             285

ACG GAG GTG ATC GCC TAC ACG CAC TGC TGT GTC AAC CCA 894
Thr Glu Val Ile Ala Tyr Thr His Cys Cys Val Asn Pro
                290             295

GTG ATC TAC GCC TTC GTT GGT GAG AGG TTC CGG AAG TAC 933
Val Ile Tyr Ala Phe Val Gly Glu Arg Phe Arg Lys Tyr
    300             305             310

CTG CGG CAG TTG TTC CAC AGG CGT GTG GCT GTG CAC CTG 972
Leu Arg Gln Leu Phe His Arg Arg Val Ala Val His Leu
        315             320

GTT AAA TGG CTC CCC TTC CTC TCC GTG GAC AGG CTG GAG 1011
Val Lys Trp Leu Pro Phe Leu Ser Val Asp Arg Leu Glu
325             330             335

AGG GTC AGC TCC ACA TCT CCC TCC ACA GGG GAG CAT GAA 1050
Arg Val Ser Ser Thr Ser Pro Ser Thr Gly Glu His Glu
        340             345             350

CTC TCT GCT GGG TTC TGACT CAGACCATAG GAGGCCAACC 1090
Leu Ser Ala Gly Phe
                355


CAAAATAAGC AGGCGTGACC TGCCAGGCAC ACTGACCAGC AGCCTGGCTC 1140


TCCCAGCCAG GTTCTGACTC TTGGCACAGC ATGGAGTCCG CCTCTTGGAT 1190
```

AGAGAGGAAT GTAATGGTGG CCTGGGGCTT CTGAGGCTTC TGGGCTTGAG 1240

TCTTTTCCAT GAACTTCTCC CCTGGTAGAA AAGAAGATGA ATGAGCAAAA 1290

CCAAATATTC CAGAGACTGG GACTAAGTGT ACCAGAGAAG GGCTTGGACT 1340

CAAGCAAGAT TTCAGATTTG TGACCATTAG CATTTGTCAA CAAAGTCACC 1390

CACTTCCCAC TATTGCTTGC ACAAACCAAT TAAACCCAGT AGTGGTGACT 1440

GTGGGCTCCA TTCAAAGTGA GCTCCTAAGC CATGGGAGAC ACTGATGTAT 1490

GAGGA 1495

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Asn Leu Ala Xaa Ala Asp Xaa Xaa
 1   5   9

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asn Pro Xaa Xaa Tyr Xaa Xaa Xaa Gly Gln
 1   5   10

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

70

Asp Arg Tyr Leu Ala Ile Val His Ala
1   5   9

WE CLAIM:

1.   An isolated C-C CKR-1 polypeptide.

2.   A composition comprising the C-C CKR-1 of claim 1,
which composition is free of a contaminating polypeptide of
the animal species that is the source of C-C CKR-1
polypeptide.

3.   An antibody that is capable of binding C-C CKR-1
polypeptide of claim 1 that does not cross-react with a known
chemokine receptor.

4.   An isolated nucleic acid molecule encoding a C-C
CKR-1 polypeptide.

5.   The nucleic acid molecule of claim 4 which is DNA
and contains greater than about 25 bases.

6.   The nucleic acid molecule of claim 4 further
comprising a promoter operably linked to the nucleic acid
sequence.

7.   The nucleic acid molecule of claim 6, wherein the
promoter is heterologous to the C-C CKR-1.

8.   An expression vector comprising the nucleic acid
sequence of claim 4 operably linked to control sequences
recognized by a host cell transformed with the vector.

9.   A host cell transformed with the vector of claim 8.

10.   A method of determining the presence of a C-C CKR-1
nucleic acid, comprising hybridizing nucleic acid encoding or
complementary to C-C CKR-1 nucleic acid to a test sample
nucleic acid and determining the presence of C-C CKR-1
nucleic acid.

11.   A method of amplifying a nucleic acid test sample
comprising priming a nucleic acid polymerase reaction with
nucleic acid encoding or complementary to a C-C CKR-1 nucleic
acid.

12.   A composition comprising the C-C CKR-1 polypeptide
of claim 1 and a pharmaceutically acceptable carrier.

13.   A composition comprising the antibody of claim 3
and a pharmaceutically acceptable carrier.

14.   An isolated polypeptide comprising the C-C CKR-1
polypeptide of claim 1 fused to a polypeptide heterologous to
the C-C CKR-1.

15.   A DNA isolate able to hybridize under stringent
conditions to a nucleotide sequence encoding C-C CKR-1 having
the amino acid sequence shown in Figure 1 and encoding a
polypeptide having C-C CKR-1 biological activity.

16.   The DNA isolate of claim 15 wherein the DNA isolate

is free of genomic DNA which encodes another polypeptide from the source of the DNA isolate.

17.   The DNA isolate of claim 15, wherein the DNA encodes a polypeptide having the amino acid sequence shown in Figure 1.

18.   The DNA isolate of claim 15, wherein the DNA encodes a polypeptide having at least about 50% sequence identity over the length of the polypeptide with that of the C-C CKR-1 amino acid sequence shown in Figure 1.

19.   The DNA isolate of claim 15, wherein the DNA encodes a polypeptide having at least about 60% sequence identity with that of the C-C CKR-1 amino acid sequence shown in Figure 1.

20.   The DNA isolate of claim 15 that is a cDNA sequence.

21.   The DNA isolate of claim 15 that is a genomic sequence.

22.   A method for identifying a C-C chemokine receptor comprising

    (a) designing a PCR primer corresponding to a transmembrane region of a seven-transmembrane spanning protein;

    (b) priming a PCR reaction with a cDNA substrate from a hematopoietic cell type known to respond to a C-C

chemokine;

    (c) recovering PCR products from step (b).

23.   The method of claim 22, wherein the primer comprises a set of degenerate oligonucleotides encoding the sequence

        LNLA(L/V)AD(L/F)(L/G).

24.   The method of claim 22, wherein the primer comprises a set of degenerate oligonucleotides encoding the sequence

        NP(I/M)(I/L)Y(A/V)(F/V)(I/M/A)GQ.

25.   The method of claim 22, wherein the primer comprises a set of degenerate oligonucleotides encoding the sequence

        DRYLAIVHA.

26.   The method of claim 22, wherein the cell type is cultured human monocytes.

27.   A method comprising transforming a host cell with DNA encoding C-C CKR-1, culturing the host cell to express the receptor on its surface, harvesting the cells, contacting the cells with a C-C chemokine variant, and determining the biological activity of the variant on the receptor.

C-C CKR-1
HUMSTSR
IL8rA
IL8rB
C5a
fMLP
hcmv-us28

FIG. 1A

4

				*
C-C CKR-1	140	FALRAR	TVTFGVITSIIIWALALASMPGLYF	SKTQWEFTHHT - - CSLHF
HUMSTSR	143	NSQRPRKL	LAEKVVYVGVWIPALLLTIPDFIFANVSEADDRYI - CDRFY
IL8rA	144	RTLTQKR - HL	VKFVCLGCWGLSMNLSLPFFLFRQAYHPNNSSPVCYEVL
IL8rB	148	RTLTQKR - YL	VKFICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDM
C5a	143	WCQNFRGAGLAWIACAVAWGLALLLTIPSFLYRVVREEYFPPKVLCGVDY
fMLP	132	WTQNHRTVSLAKKVIIGPWVMALLLTLPVII - RVTTVPGKTGTVACTFNF
hcmv-us28	136	- - MRYRPVKQACLFSIFWWIFAVIIAIPHEMVVTKKDNQ - - - - - - CMTDY

5

C-C CKR-1	188	PHESLREWKLFQ - - - - - - -	ALKLNLFGLVLPLLVMIICYTGIIKILLR
HUMSTSR	191	PNDL - - WVVVF - - - - - - -	QFQHIMVGLILPGIVILSCYCIISKLSH
IL8rA	192	GNDTAK - WRMVL - - - - - -	RILPHTFGFIVPLFVMLFCYGFTLRTLFK
IL8rB	196	GNNTAN - WRMLL - - - - - -	RILPQSFGFIVPLLIMLFCYGFTLRTLFK
C5a	193	SHDKRRERAVA - - - - - - -	IVRLVLGFLWPLLTLTICYTFILLRTWS
fMLP	181	SPWTNDPKERINVAVAMLTVRGIIRFIIGFSAPMSIVAVSYGLIATKIHK
hcmv-us28	178	DYLEVS - YPIIL - - - - - -	NVELMLGAFVIPLSVISYCYYRILSRIVAV

6

C-C CKR-1	229	RPNEKKSKAVRLIFVIMIIFFLFWTPYNLTILISVFQDF - LFTHECEQSR
HUMSTSR	229	SKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFEN
IL8rA	232	AHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQVIQETCERRN
IL8rB	236	AHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQVIQETCERRN
C5a	232	RRATRSTKTLKVVVAVVASFFIFWLPYQVTGIM - MSFLEPSSPTF - - - L
fMLP	231	QGLIKSSRPLRVLSFVAAFFLCWSPYQVVALIATVRIRELLQGMY - - - K
hcmv-us28	218	SQSRHKGRIVRVLIAVVLVFIIFWLPYHLTLFVDTLKLLKWSSCEFER

FIG. IB

FIG. IC

C-C CKR-1	278	HLDLA	VQVTE	VIAYT	HCCVN	PVLYA	FVGER	FRKYL	RQLFH RRVAV HLVKW
HUMSTSR	279	TVHKW	ISITE	ALAFF	HCCLN	PILYA	FLGAK	FKTSA	QHALT SVSRG SLKI
IL8rA	282	NIGRA	LDATE	ILGFL	HSCLN	PIIYA	FIGQN	FRHGF	LKILA MHGLV SKEFL
IL8rB	286	HIDRA	LDATE	ILGIL	HSCLN	PLIYA	FIGQK	FRHGL	KILAI HGLIS KDSL
C5a	277	LLNKL	DSLCV	SFAYI	NCCIN	PIILY	VVAGQ	GFQGR	LRKSL PSLLR NVLTEE
fMLP	278	EIGFA	VDVTS	ALAFF	NSCLN	PMLYV	FMGQD	FRERL	IHALP ASLER ALTED
hcmv-us28	268	SLKRA	LILTE	SLAFC	HCCLN	PLLYV	FVGTK	FRKNY	TVCWP SFASD SFPAM
Towne-us28	300								QELHCLLAEFRQRLFSRD

C-C CKR-1	328	LPFLS	VDRLE RVSST	SPSTGEHELSAGF
HUMSTSR	329	LSKGK	RGGHS SVSTE	SESSFHSS
IL8rA	332	ARHRV	TSYTS SSVNV	SSNL
IL8rB	336	PKDSR	PSFVG SSGHT	STTL
C5a	327	SVVRE	SKSFT RSTVD	TMAQKTQAV
fMLP	328	S-TQT	SDTAT NSTLP	SAEVELQAK
hcmv-us28	318	YPGTTA		

FIG. 2
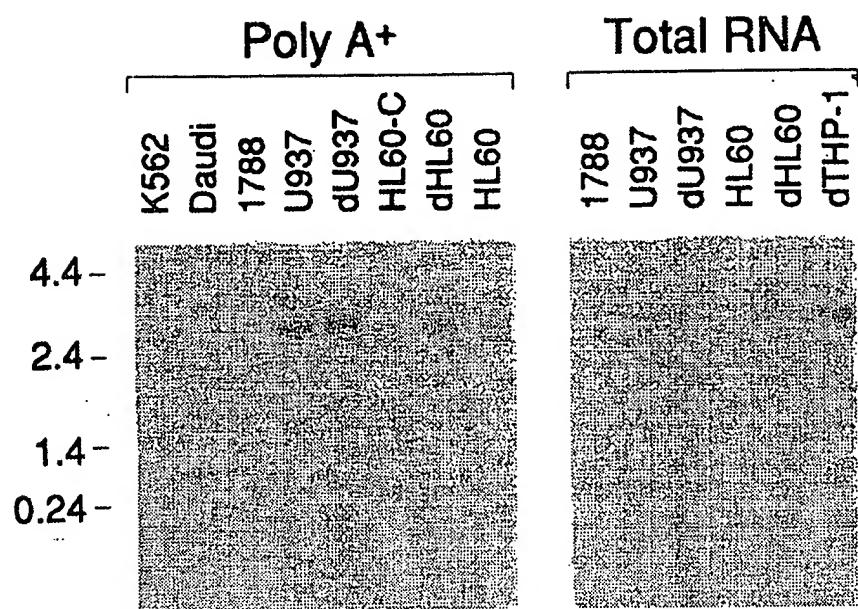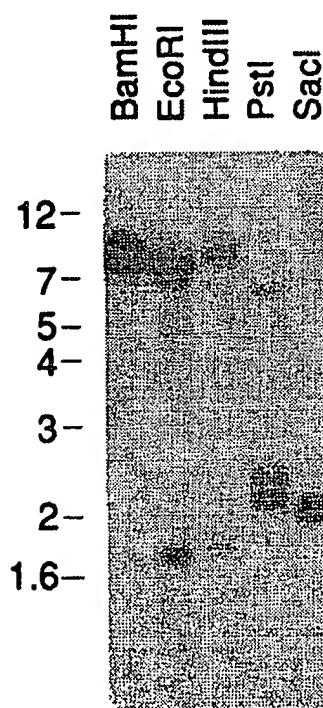
FIG. 3A

FIG. 3B

6/13

FIG. 4A

FIG. 4B

7/13

FIG. 5B

FIG. 5D

FIG. 5A

FIG. 5C

8/13

## FIG. 6A

FIG. 6B

FIG. 7
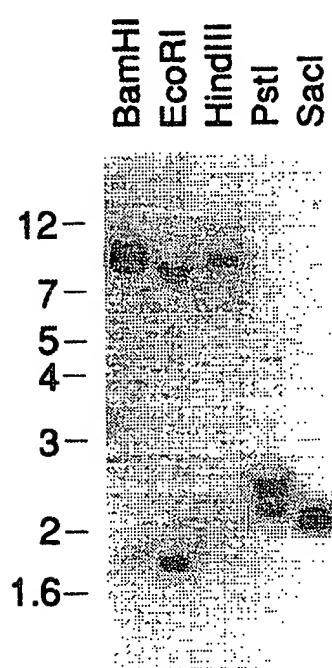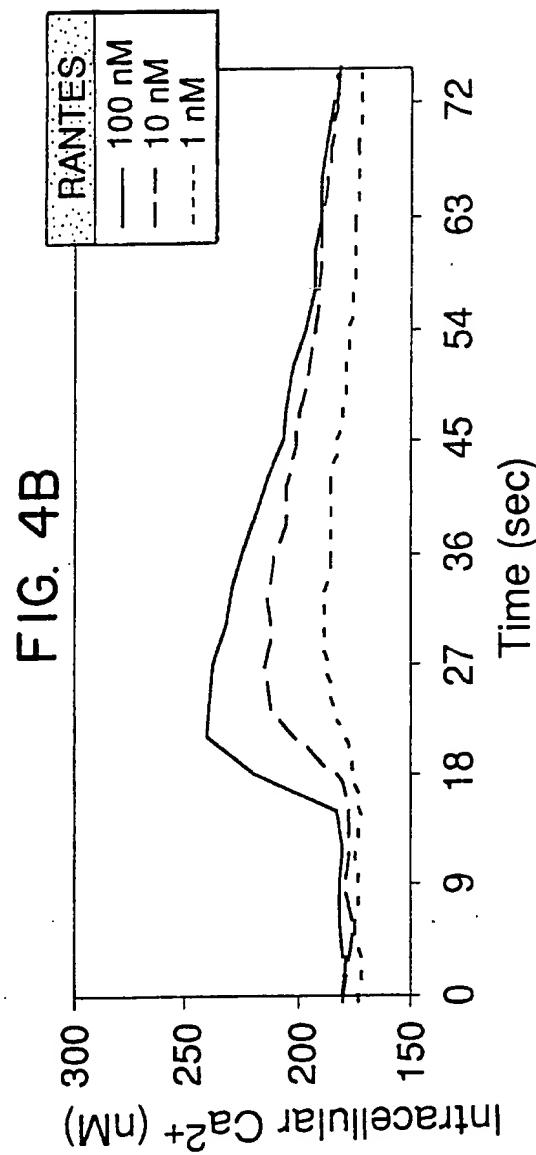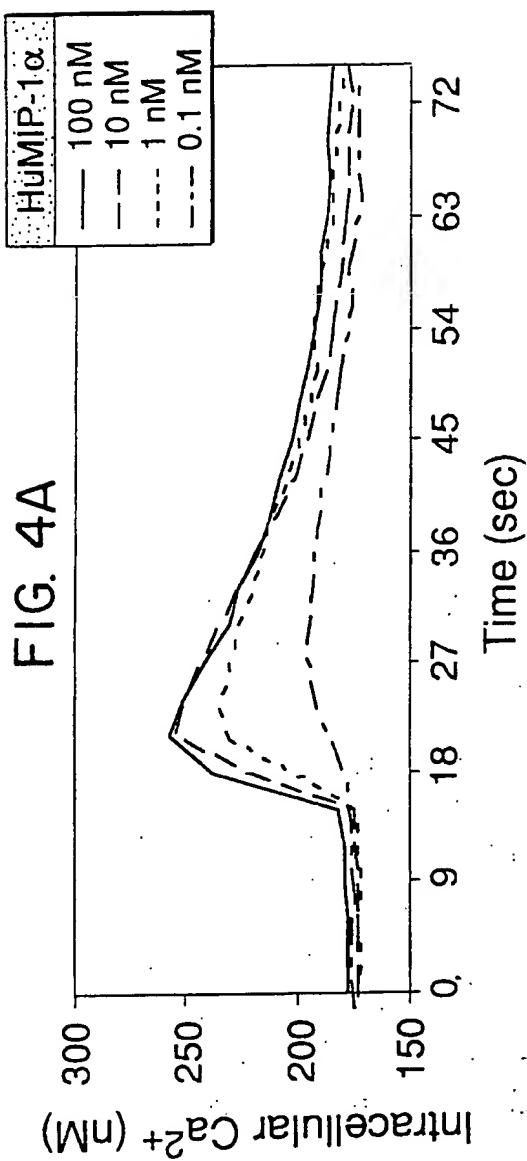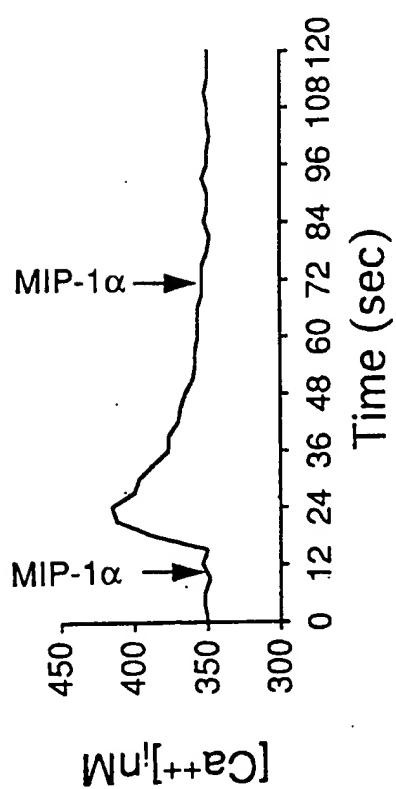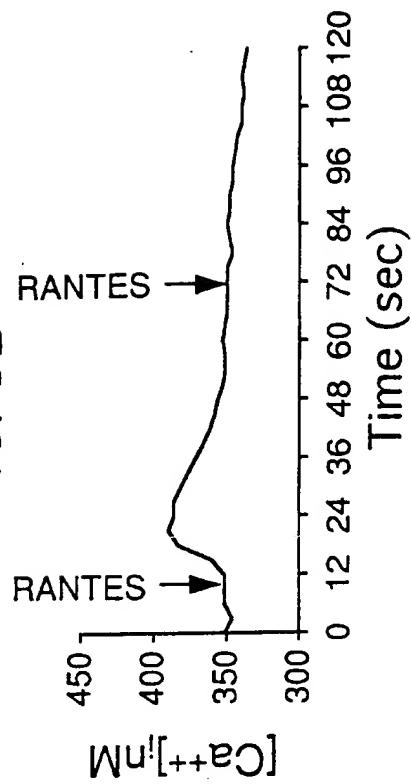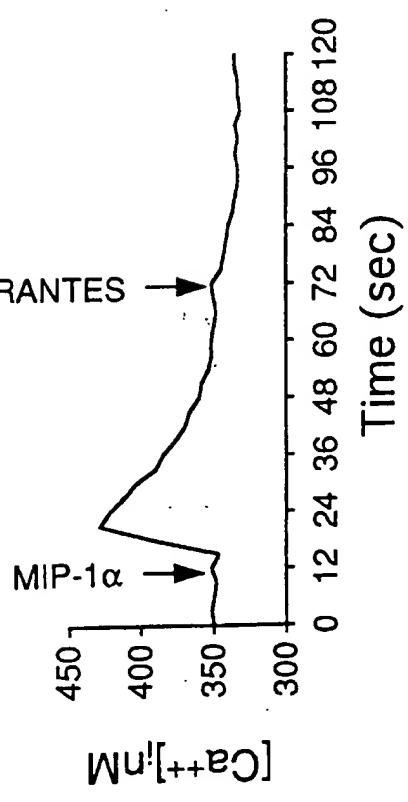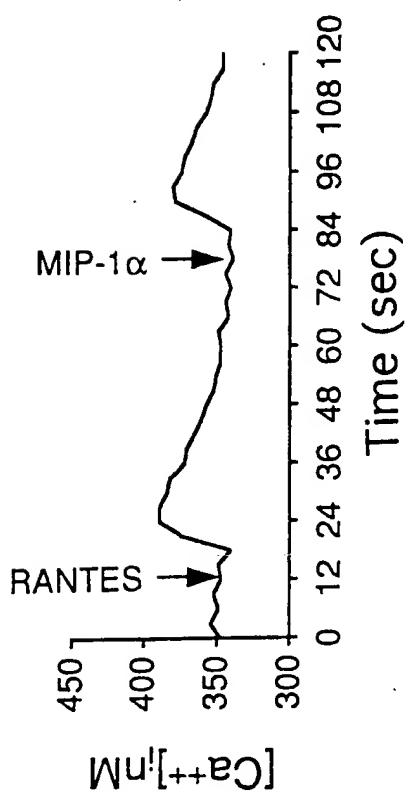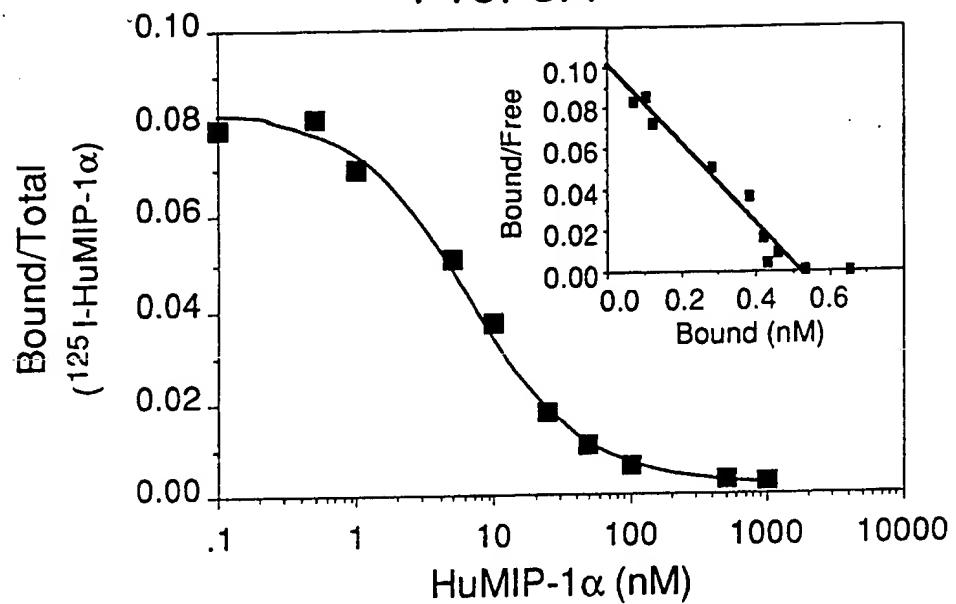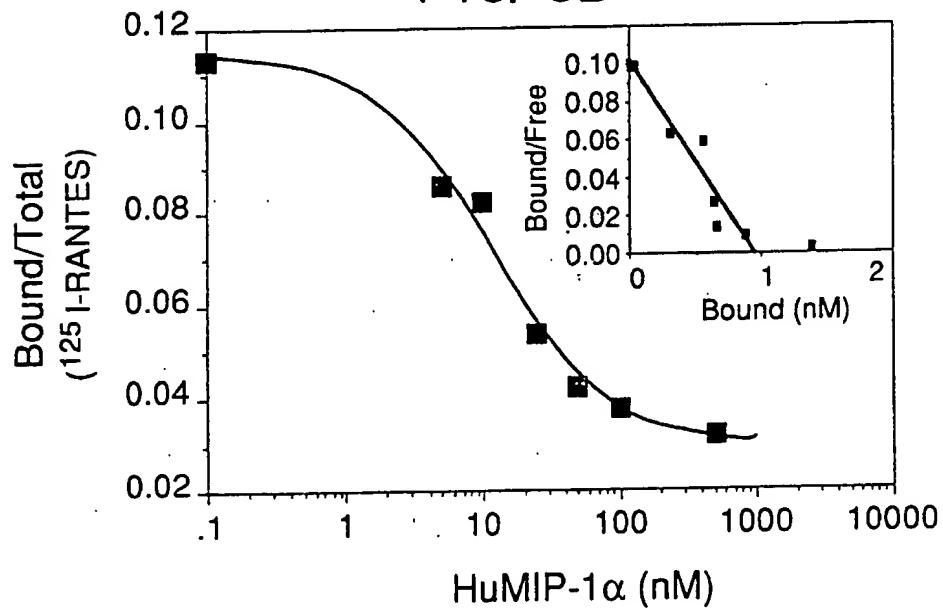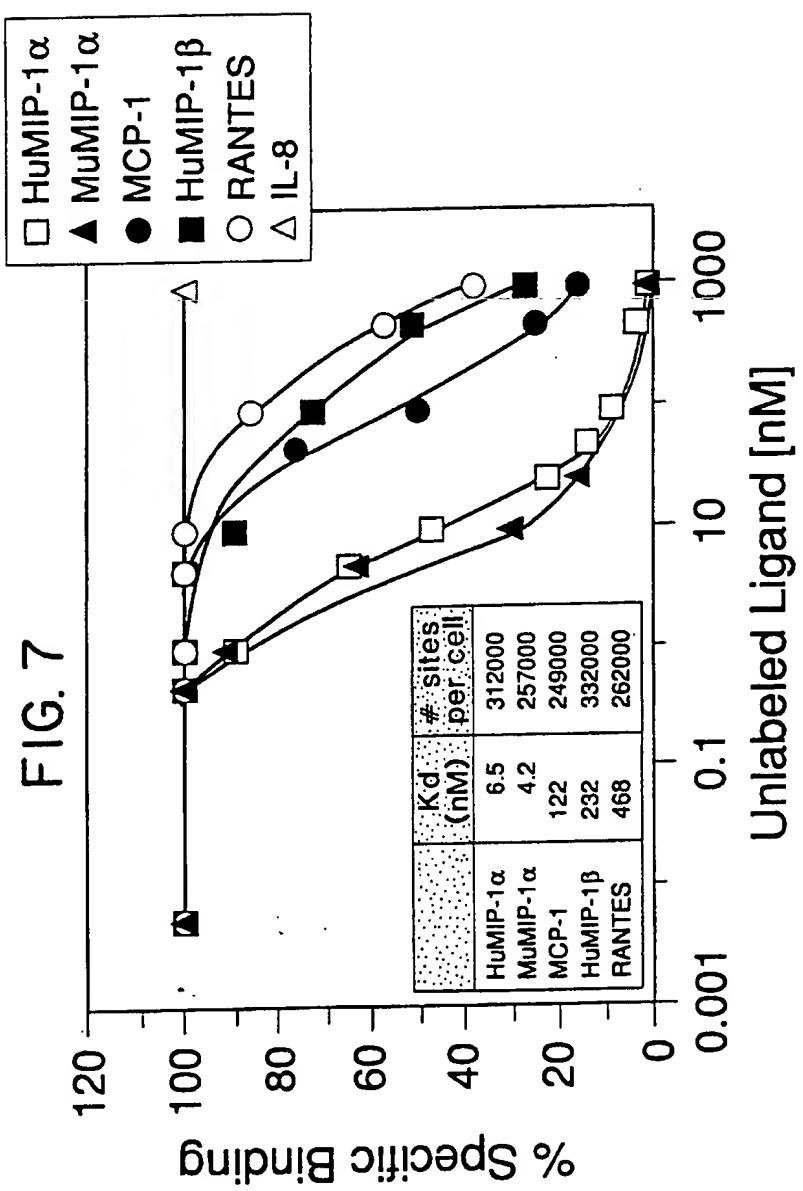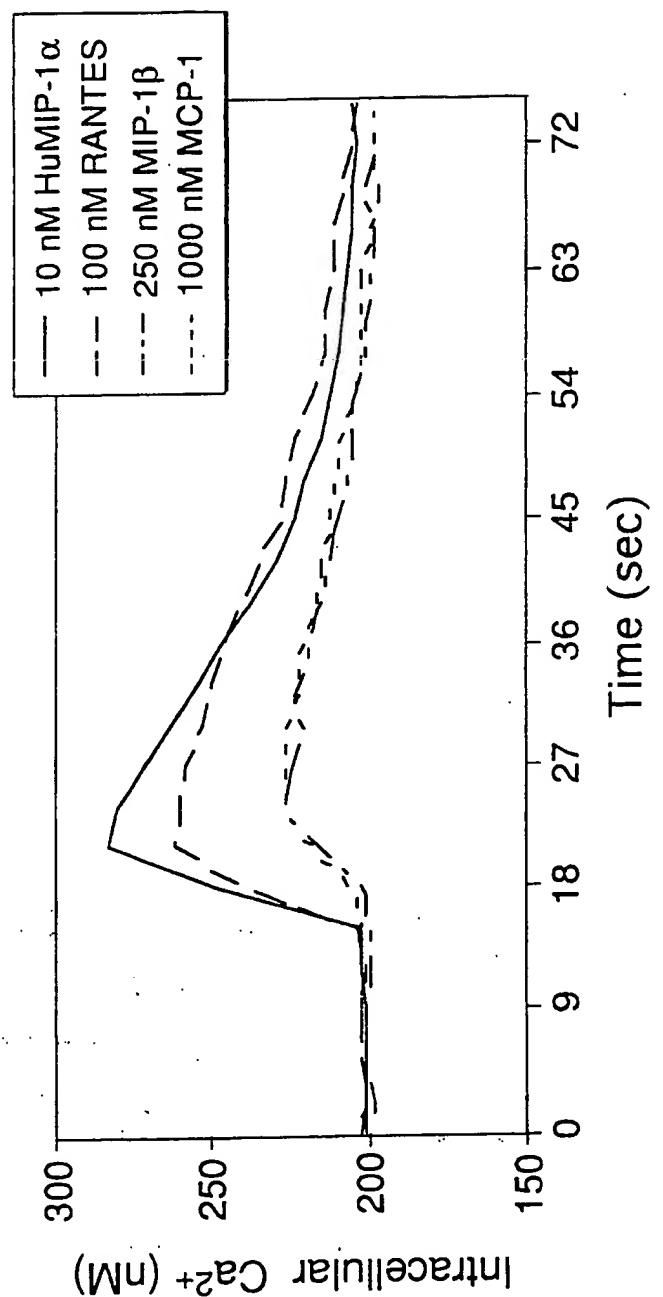
FIG. 8

11/13

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  1 ATGGAAACTC CAAACACCAC AGAGGACTAT GACACGACCA CAGAGTTTGA
    TACCTTTGAG GTTTGTGGTG TCTCCTGATA CTGTGCTGGT GTCTCAAACT
  1 M  E  T  P   N  T  T    E  D  Y    D  T  T    E  F  D

    CTATGGGGAT GCAACTCCGT GCCAGAAGGT GAACGAGAGG GCCTTTGGGG
    GATACCCCTA CGTTGAGGCA CGGTCTTCCA CTTGCTCTCC CGGAAACCCC
    Y  G  D    A  T  P  C   Q  K  V    N  E  R    A  F  G  A

101 CCCAACTGCT GCCCCCTCTG TACTCCTTGG TATTTGTCAT TGGCCTGGTT
    GGGTTGACGA CGGGGGAGAC ATGAGGAACC ATAAACAGTA ACCGGACCAA
 35 Q  L  L    P  P  L    Y  S  L  V   F  V  I    G  L  V

    GGAAACATCC TGGTGGTCCT GGTCCTTGTG CAATACAAGA GGCTAAAAAA
    CCTTTGTAGG ACCACCAGGA CCAGGAACAC GTTATGTTCT CCGATTTTTT
    G  N  I  L   V  V  L    V  L  V    Q  Y  K  R   L  K  N

201 CATGACCAGC ATCTACCTCC TGAACCTGGC CATTTCTGAC CTGCTCTTCC
    GTACTGGTCG TAGATGGAGG ACTTGGACCG GTAAAGACTG GACGAGAAGG
 68 M  T  S    I  Y  L  L   N  L  A    I  S  D    L  L  F  L

    TGTTCACGCT TCCCTTCTGG ATCGACTACA AGTTGAAGGA TGACTGGGTT
    ACAAGTGCGA AGGGAAGACC TAGCTGATGT TCAACTTCCT ACTGACCCAA
    F  T  L    P  F  W    I  D  Y  K   L  K  D    D  W  V

301 TTTGGTGATG CCATGTGTAA GATCCTCTCT GGGTTTTATT ACACAGGCTT
    AAACCACTAC GGTACACATT CTAGGAGAGA CCCAAAATAA TGTGTCCGAA
101 F  G  D  A   M  C  K    I  L  S    G  F  Y  Y   T  G  L

    GTACAGCGAG ATCTTTTTCA TCATCCTGCT GACGATTGAC AGGTACCTGG
    CATGTCGCTC TAGAAAAAGT AGTAGGACGA CTGCTAACTG TCCATGGACC
    Y  S  E    I  F  F  I   I  L  L    T  I  D    R  Y  L  A

401 CCATCGTCCA CGCCGTGTTT GCCTTGCGGG CACGGACCGT CACTTTTGGT
    GGTAGCAGGT GCGGCACAAA CGGAACGCCC GTGCCTGGCA GTGAAAACCA
135 I  V  H    A  V  F    A  L  R  A   R  T  V    T  F  G

    GTCATCACCA GCATCATCAT TTGGGCCCTG GCCATCTTGG CTTCCATGCC
    CAGTAGTGGT CGTAGTAGTA AACCCGGGAC CGGTAGAACC GAAGGTACGG
    V  I  T  S   I  I  I    W  A  L    A  I  L  A   S  M  P

501 AGGCTTATAC TTTTCCAAGA CCCAATGGGA ATTCACTCAC CACACCTGCA
    TCCGAATATG AAAAGGTTCT GGGTTACCCT TAAGTGAGTG GTGTGGACGT
168 G  L  Y    F  S  K  T   Q  W  E    F  T  H    H  T  C  S

    GCCTTCACTT TCCTCACGAA AGCCTACGAG AGTGGAAGCT GTTTCAGGCT
    CGGAAGTGAA AGGAGTGCTT TCGGATGCTC TCACCTTCGA CAAAGTCCGA
    L  H  F    P  H  E    S  L  R  E   W  K  L    F  Q  A

601 CTGAAACTGA ACCTCTTTGG GCTGGTATTG CCTTTGTTGG TCATGATCAT
    GACTTTGACT TGGAGAAACC CGACCATAAC GGAAACAACC AGTACTAGTA
201 L  K  L  N   L  F  G    L  V  L    P  L  L  V   M  I  I

    CTGCTACACA GGGATTATAA AGATTCTGCT AAGACGACCA AATGAGAAGA
    GACGATGTGT CCCTAATATT TCTAAGACGA TTCTGCTGGT TTACTCTTCT
    C  Y  T    G  I  I  K   I  L  L    R  R  P    N  E  K  K
```

FIG. 9A

12/13

```
701 AATCCAAAGC TGTCCGTTTG ATTTTTGTCA TCATGATCAT CTTTTTTCTC
    TTAGGTTTCG ACAGGCAAAC TAAAAACAGT TGTACTAGTA GAAAAAAGAG
235  S  K  A   V  R  L    I  F  V  I   M  I  I    F  F  L

    TTTTGGACCC CCTACAATTT GACTATACTT ATTTCTGTTT TCCAAGACTT
    AAAACCTGGG GGATGTTAAA CTGATATGAA TAAAGACAAA AGGTTCTGAA
     F  W  T  P   Y  N  L    T  I  L    I  S  V  F   Q  D  F

801 CCTGTTCACC CATGAGTGTG AGCAGAGCAG ACATTTGGAC CTGGCTGTGC
    GGACAAGTGG GTACTCACAC TCGTCTCGTC TGTAAACCTG GACCGACACG
268  L  F  T   H  E  C  E   Q  S  R    H  L  D    L  A  V  Q

    AAGTGACGGA GGTGATCGCC TACACGCACT GCTGTGTCAA CCCAGTGATC
    TTCACTGCCT CCACTAGCGG ATGTGCGTGA CGACACAGTT GGGTCACTAG
      V  T  E   V  I  A    Y  T  H  C   C  V  N    P  V  I

901 TACGCCTTCG TTGGTGAGAG GTTCCGGAAG TACCTGCGGC AGTTGTTCCA
    ATGCGGAAGC AACCACTCTC CAAGGCCTTC ATGGACGCCG TCAACAAGGT
301  Y  A  F  V   G  E  R    F  R  K    Y  L  R  Q    L  F  H

    CAGGCGTGTG GCTGTGCACC TGGTTAAATG GCTCCCCTTC CTCTCCGTGG
    GTCCGCACAC CGACACGTGG ACCAATTTAC CGAGGGGAAG GAGAGGCACC
      R  R  V   A  V  H  L   V  K  W    L  P  F    L  S  V  D

1001 ACAGGCTGGA GAGGGTCAGC TCCACATCTC CCTCCACAGG GGAGCATGAA
     TGTCCGACCT CTCCCAGTCG AGGTGTAGAG GGAGGTGTCC CCTCGTACTT
335   R  L  E   R  V  S    S  T  S  P   S  T  G    E  H  E

     CTCTCTGCTG GGTTCTGACT CAGACCATAG GAGGCCAACC CAAAATAAGC
     GAGAGACGAC CCAAGACTGA GTCTGGTATC CTCCGGTTGG GTTTTATTCG
      L  S  A  G   F

1101 AGGCGTGACC TGCCAGGCAC ACTGACCAGC AGCCTGGCTC TCCCAGCCAG
     TCCGCACTGG ACGGTCCGTG TGACTGGTCG TCGGACCGAG AGGGTCGGTC

     GTTCTGACTC TTGGCACAGC ATGGAGTCCG CCTCTTGGAT AGAGAGGAAT
     CAAGACTGAG AACCGTGTCG TACCTCAGGC GGAGAACCTA TCTCTCCTTA

1201 GTAATGGTGG CCTGGGGCTT CTGAGGCTTC TGGGCTTGAG TCTTTTCCAT
     CATTACCACC GGACCCCGAA GACTCCGAAG ACCCGAACTC AGAAAAGGTA

     GAACTTCTCC CCTGGTAGAA AAGAAGATGA ATGAGCAAAA CCAAATATTC
     CTTGAAGAGG GGACCATCTT TTCTTCTACT TACTCGTTTT GGTTTATAAG

1301 CAGAGACTGG GACTAAGTGT ACCAGAGAAG GGCTTGGACT CAAGCAAGAT
     GTCTCTGACC CTGATTCACA TGGTCTCTTC CCGAACCTGA GTTCGTTCTA

     TTCAGATTTG TGACCATTAG CATTTGTCAA CAAAGTCACC CACTTCCCAC
     AAGTCTAAAC ACTGGTAATC GTAAACAGTT GTTTCAGTGG GTGAAGGGTG

1401 TATTGCTTGC ACAAACCAAT TAAACCCAGT AGTGGTGACT GTGGGCTCCA
     ATAACGAACG TGTTTGGTTA ATTTGGGTCA TCACCACTGA CACCCGAGGT

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     AAGTTTCACT CGAGGATTCG GTACCCTCTG TGACTACATA CTCCT
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FIG. 9B
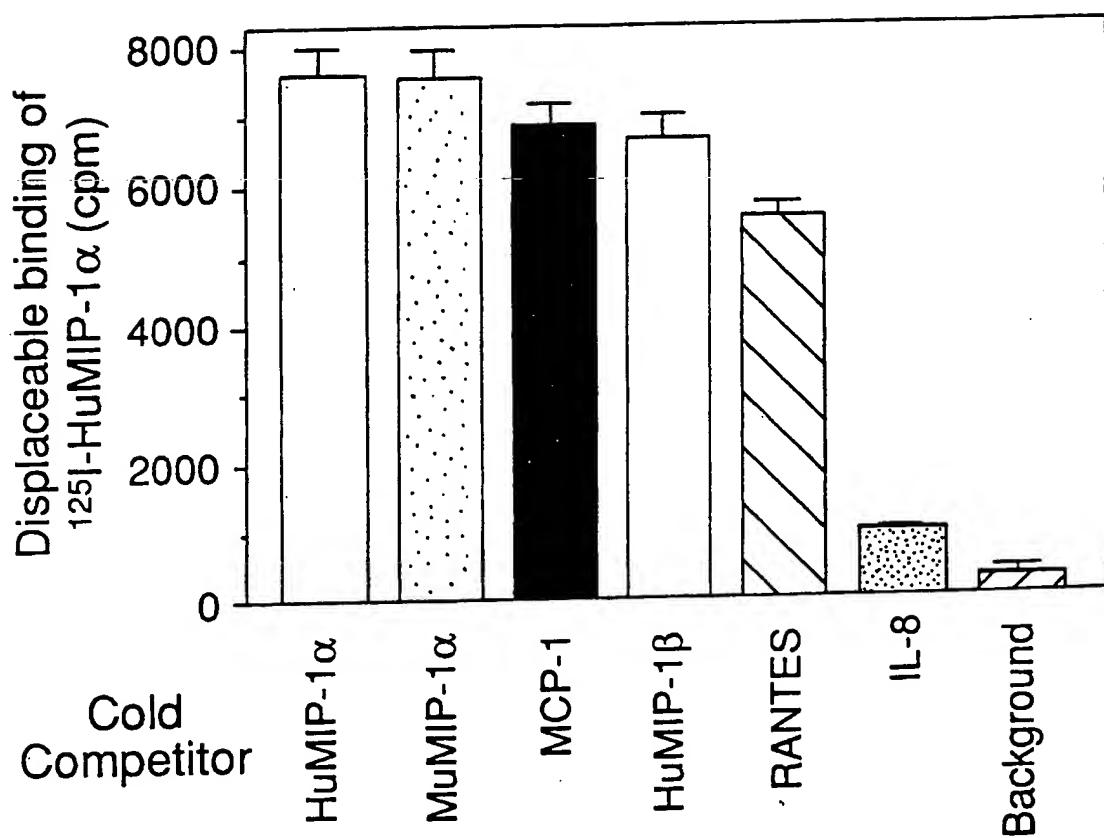
13/13

FIG. 10

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/12 C07K13/00 C12P21/08 C12N5/10 C12N15/62
A61K37/02 A61K39/395 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N C07K C12P C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CELL vol. 72 , 12 February 1993 , CAMBRIDGE, NA US pages 415 - 425 NEOTE K;DIGREGORIO D;MAK JY;HORUK R;SCHALL TJ 'Molecular cloning, functional expression, and signaling' see the whole document ---	1-27
Y	WO,A,92 17497 (GENENTECH, INC. , US) 15 October 1992 see claims 8,15 ---	22-26
Y	WO,A,92 18641 (THE TRUSTEES OF BOSTON UNIVERSITY, US) 29 October 1992 see page 11, line 8 - page 12, line 12 -----	22-26

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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'P' document published prior to the international filing date but later than the priority date claimed

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'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 1994	01. 03. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nauche, S

Form PCT/ISA/210 (second sheet) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9217497	15-10-92	EP-A-	0577752	12-01-94
WO-A-9218641	29-10-92	CA-A-	2107682	11-10-92
		EP-A-	0579739	26-01-94